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Minireview

Non-invasive assessment of the effects of iontophoresis on human skin in-vivo

Catherine Curdy, Yogeshvar N. Kalia and Richard H. Guy

Abstract

The stratum corneum (SC), the outermost layer of the skin, presents a formidable barrier to transdermal drug delivery. As a result, different strategies have been developed to enhance drug transport into and through skin. Iontophoresis involves the application of a small electrical current which drives molecules across the skin and controls relatively well the rate of delivery. Although the technique has been widely investigated in-vitro, the evaluation of skin integrity in-vivo after iontophoresis is absolutely necessary for the future clinical application of this approach. This paper reviews the non-invasive biophysical techniques which have been used to assess the effects of current application on human skin in-vivo. Specifically, transepidermal water loss, infrared spectroscopy, impedance spectroscopy and skin blood flow measurements are discussed. After first presenting the basic principles of these methods, their application to the determination of SC barrier function and skin integrity is addressed, and the criteria for selecting the most appropriate approach are considered.

Centre Interuniversitaire de Recherche et d'Enseignement, Universities of Geneva and Lyon, 'Pharmapeptides', F-74166 Archamps, France.

Catherine Curdy, Yogeshvar N. Kalia, Richard H. Guy

Faculté des Sciences, Section de Pharmacie, Laboratoire de Pharmacie Galénique, Université de Genève, CH-1211 Genève 4, Switzerland.

Catherine Curdy, Yogeshvar N. Kalia, Richard H. Guy

Correspondence: R. H. Guy, Centre Interuniversitaire de Recherche et d'Enseignement, Universities of Geneva and Lyon, 'Pharmapeptides', Parc d'Affaires International, F-74166 Archamps, France. E-mail: rhg@pharma1.cur-archamps.fr

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Introduction

The barrier function of the skin resides in the most superficial layer of the epidermis, the stratum corneum (SC) (Elias 1991). The principal role of the SC is to limit water loss from the body (Potts & Francoeur 1990). The excellent barrier function of the SC means that it is also a major resistance to transdermal drug delivery (TDD), such that the number of molecules successfully delivered by this route is limited. However, the advantages of TDD (e.g. avoidance of the first-pass effect, reduced inter- and intra-subject variability, reduced frequency of drug dosing, improved compliance) have provoked the examination of numerous strategies to improve and enhance drug transport across the skin (Guy 1996; Merino et al 1997a). Among these, iontophoresis has been quite intensively studied in the last 20 years (Glickfeld et al 1989; Rao et al 1995; Tamada et al 1995; Merino et al 1997b; Guy 1998) and practical devices (both for delivery and for non-invasive monitoring of blood glucose) are nearing commercialization (Tamada et al 1999). Iontophoresis involves the application of a small electrical current which can significantly improve the permeation of both charged and very polar (yet electrically neutral) species across the membrane (Green et al 1993; Sage 1995; Singh et al 1999). The delivery of simple drugs such as lidocaine and fentanyl has been well-established (Singh & Roberts 1993; Ashburn et al 1995) and the potential to administer more challenging peptides, such as luteinizing hormone-releasing hormone analogues, somatostatin, calcitonin and parathyroid hormone, has been demonstrated (Delgado-Charro & Guy 1998) in-vivo either in man or a relevant animal model, such as the pig.

It has become necessary, as a result of this activity, to address whether iontophoresis itself elicits specific effects (reversible or irreversible) on the skin, such as perturbation of barrier function, disruption of the lipid–protein structure of the SC, or local irritation or even sensitization. The impact of key iontophoretic formulation parameters, including current profile, density and time of application, the electrolyte composition and pH of the electrode solutions or gels and the nature of the drug itself, have to be considered. Clearly, the chronic use of iontophoresis, either for drug delivery or for non-invasive monitoring purposes, demands that the method be well tolerated and tolerable. In this article, we review the most widely applied biophysical techniques for the evaluation of the effects of iontophoresis on the skin, and consider the literature with respect to the response and recovery of barrier function after iontophoresis.

Non-invasive biophysical techniques to assess skin integrity in-vivo

Transepidermal water loss (TEWL)

The excellent barrier function of the SC limits the insensible, passive rate of water loss across the skin to about 5 g m^{-2} h^{-1} under normal conditions. Perturbations to the SC, induced by a variety of means (tape-stripping, UV radiation, disease, corrosive chemicals, etc.), invariably cause TEWL to increase (Van der Walk & Maibach 1990), such that the parameter is routinely used to assess skin barrier integrity. The apparatus used to determine TEWL, the Evaporimeter (ServoMed, Stockholm, Sweden) or the Tewameter (Courage-Khazaka Electronic GmbH, Cologne, Germany), operate by measuring the water vapour pressure gradient above the SC and then back-calculating the rate at which water is exiting the SC (Nilsson 1977; Barel & Clarys 1995; Pinnagoda & Tupker 1995) (Figure 1). Measurements are sensitive to temperature and relative humidity and are therefore most usefully performed under carefully controlled conditions (Pinnagoda et al 1990).

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy

The attenuated total reflectance technique allows IR spectra of human skin to be recorded rapidly, in-vivo (Figure 2). The site typically studied is the ventral forearm which is positioned on the internal reflectance element; within this crystal, multiple internal reflections of the infrared beam take place and the evanescent wave is absorbed by the SC at the interface with the skin (Puttnam 1972). The ATR-FTIR method has been discussed in detail elsewhere (Markovich & Pidgeon 1991; Naik & Guy 1997), and therefore attention is focussed here on the components of the stratum corneum which absorb in the IR range and which may be used to report on the barrier function, and by implication, on the effects of iontophoresis.

The methylene group symmetric and asymmetric stretching vibrations at 2850 cm^{-1} and 2920 cm^{-1}, respectively, have been most widely exploited (Potts & Francoeur 1993). The frequencies of these absorbances report directly on the conformational order of the SC intercellular lipid domains. Disordering the lipids (e.g. by increasing temperature or by the application of penetration enhancers) causes the frequencies of these vibrations to increase (Knutson et al 1985). In turn, such effects have been correlated with increased drug and water permeation across the SC (Golden et al 1987). Furthermore, when used in conjunction with progressive tape-stripping of the SC, the ATR-FTIR spectrum in this region has revealed that the lipids are more ordered in the deeper SC than near the surface (Bommannan et al 1990). Other significant absorbances are the amide I

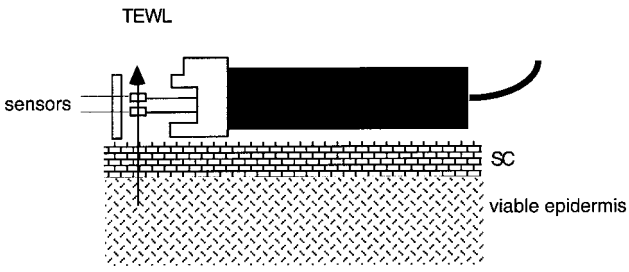

Figure 1 Schematic diagram of the measurement of transepidermal water loss (TEWL). Sensors at specific distances from the stratum corneum (SC) surface detect a water gradient from which TEWL is determined (redrawn from Pinnagoda & Tupker 1995).

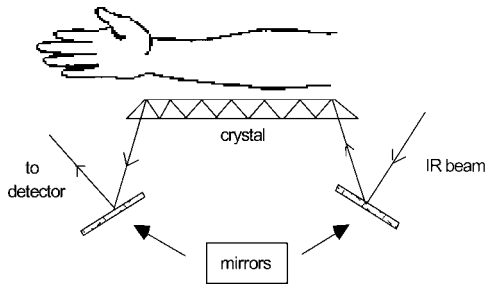

Figure 2 Attenuated total reflectance-Fourier transform infrared spectroscopy of human stratum corneum in-vivo (redrawn from Green & Hadgraft 1993).

and amide II bands centred at about 1640 cm^{-1} and 1565 cm^{-1}, respectively. Although deconvolution of the conformational information related to SC keratin contained in these absorbances is not straightforward (Brand et al 1997), changes in these peaks have been related to changes in the level of SC hydration (Bommannan et al 1990). The latter can also be probed by the appearance of a weak O-H absorbance at around 2100 cm^{-1} (i.e. in a region of the SC usually quite transparent) (Potts et al 1985).

Impedance spectroscopy (*IS*)

The SC is also the skin's principal barrier to the flow of an electrical current (i.e. to the movement of ions across the membrane). The skin has been variously modelled by different electrical circuits (Figure 3), such as a resistor and a capacitor in parallel (Tregear 1966; Yamamoto & Yamamoto 1976), this same parallel-RC model in series with another resistance (De Nuzzio & Berner 1990), or more complex models with additional resistances and capacitances or with the replacement of the capacitance by a constant-phase element, designed to take into account the non-conformity of the SC with simpler equivalent circuits (Kontturi et al 1993; Kontturi & Murtomäki 1994). Physically, the resistive component of the parallel-RC model has been associated with 'shunt' pathways across the SC (e.g. hair follicles, sweat glands), and the capacitive part is linked to the lipid–protein matrix of the membrane; the added resistance in series has been attributed to those parts of the skin, other than the SC (viable tissue, etc.) which can also restrict, albeit to a lesser extent, the flow of ions. The basis for the different equivalent circuits of the skin

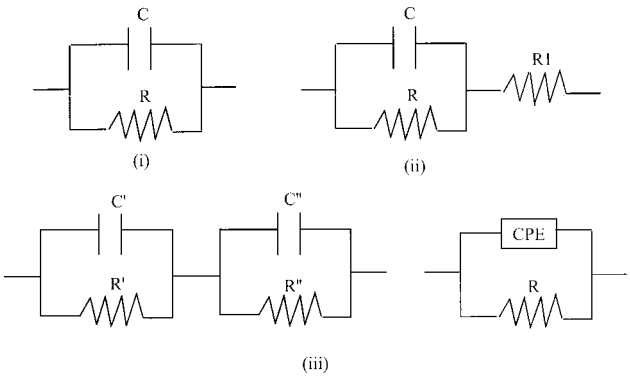

Figure 3 Electrical circuit models of the skin: (i) a resistor and a capacitor in parallel; (ii) a parallel-RC circuit with an additional resistance in series; and (iii) more complex models, such as two parallel-RC circuits in series, or a constant-phase element (CPE) and a resistor in parallel.

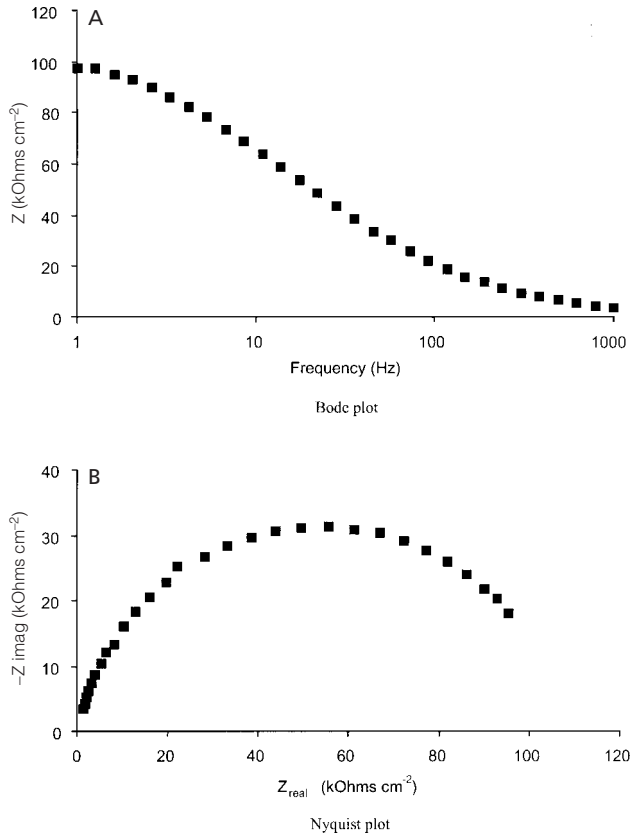

Figure 4 Common representations of skin impedance data. A. Bode plot of impedance vs frequency. B. Nyquist plot of the imaginary component vs real part of the impedance. The data shown are measured impedance values 30 min after iontophoresis (0.1 mA cm^{-2} for 15 min) of a human volunteer.

are experiments using impedance spectroscopy, whereby the impedance of the tissue to the flow of a small alternating current is measured as a function of frequency. Fitting the resulting spectrum to the one or other equivalent circuit model enables the characteristic resistive and capacitive components to be deduced. Data are usually represented in the form of either a Bode (impedance vs frequency) or a Nyquist plot (imaginary component of the impedance vs the real part) (Burnette & De Nuzzio 1997) (Figure 4). Clearly, as the barrier function of the SC is reduced (e.g. by tape-stripping) (Yamamoto & Yamamoto 1976; Kalia & Guy 1995), the form of the impedance spectrum changes accordingly, and the results may be considered complementary to those obtained, for example, by TEWL (Kalia et al 1996b). The potential use of IS to assess the impact of iontophoresis on SC barrier function is evident, given that the technique is sensitive to the movement of ions through the tissue.

Laser Doppler flowmetry (or velocimetry) (LDF)

LDF is a method used to measure non-invasively the relative skin blood flow in-vivo. Radiation at 632.9 nm from a helium-neon laser is transmitted to the skin via an optical fibre. The light enters the tissue and is reflected both at the same frequency as the incident source by stationary tissue components and at a (Doppler) shifted frequency by erythrocytes which are moving through the cutaneous microcirculation (Bircher 1995). The latter signal is proportional to the averaged product of the number and the velocity of the red blood cells in motion beneath the site of observation. The use of LDF in various applications (e.g. tissue viability subsequent to a burn, peripheral vascular disease, diminished microcirculatory function in diabetics, response to vaso-active topically-applied drugs, reaction to UV exposure, etc.) has been reported (Tur 1999), and its value for the assessment of detrimental effects to the skin subsequent to a penetration enhancement strategy is clear.

Evaluation of skin barrier function post-iontophoresis

TEWL measurements

Although iontophoresis for 30 min at 0.2 mA cm^{-2} rapidly and significantly increased TEWL (Préat et al 1993) at both the anode and cathode (compared with the control site where the skin was exposed to the same electrolyte solution used without current), more recent experiments have not found profound changes in TEWL after current application. For example, although TEWL was higher after current passage (0.5 mA cm^{-2} for 5, 10 and 25 min), this change was no different from that at control sites (i.e. no current applied), suggesting that TEWL increased simply as a result of skin hydration after exposure to the electrolyte solution applied (Brand et al 1997; Van der Geest et al 1996) (Figure 5). Furthermore, Kalia et al (1996a) reported that TEWL was not significantly affected (again compared with the no-current control) by the application of either 0.1 mA cm^{-2} or 0.3 mA cm^{-2} for 15 min and was independent of electrode polarity.

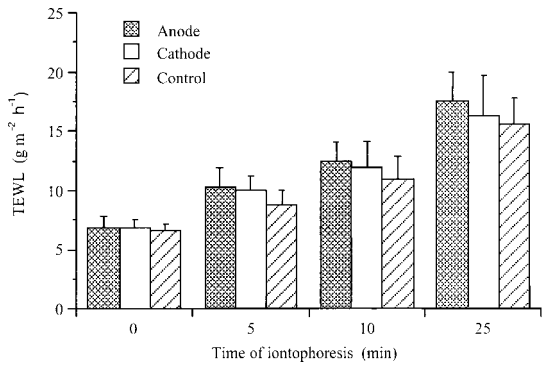

Figure 5 Transepidermal water loss (TEWL) measurements recorded 15 min after 5, 10 or 25 min current passage (0.5 mA cm^{-2}). The basal, control values before iontophoresis (t = 0) are shown for comparison (mean ± s.d., n = 7) (redrawn from Brand et al 1997).

ATR-FTIR spectroscopy

There are limited reports in the literature on the effects of iontophoresis on the reflectance IR spectrum of human SC in-vivo. The data reported (Green & Hadgraft 1993; Brand et al 1997) reveal, in general, no significant changes in the conformational order of the lipids within the intercellular domains. Equally, despite application of deconvolution methods to examine whether protein conformational alterations could be deduced from the amide I and amide II absorbances, no consistent or significant impact of iontophoresis was found (Brand et al 1997). As expected, IR spectroscopy has revealed increased SC hydration (through the O-H absorbance at about 2100 cm^{-1}) when exposed to an electrolyte buffer (Green & Hadgraft 1993); however, the increase seen was no different whether current was applied or not.

IS evaluation

The low-frequency impedance of human skin (effectively its electrical resistance) is usually high (approx. 1 MΩ cm^{-2}). Passage of an iontophoretic current causes a very rapid decrease in this parameter which is sustained over the period of current application. Subsequently, the electrical properties of the barrier recover, this process being somewhat dependent on several factors including, of course, the current density applied, time of current application, and nature of the electrolyte used (Kalia & Guy 1995; Oh & Guy 1995). The sharp drop in low-frequency impedance can almost certainly be attributed to the fact that iontophoresis quickly and dramatically increases the number of ions within the skin, thereby raising the conductance of the membrane and diminishing its electrical resistance. To refer to such changes as 'skin damage', as can be found in the literature (Burnette & Ongpipattanakul 1988), is clearly an over reaction to an inevitable change, which is very easily shown to be reversible over time after termination of current passage. Furthermore, simple in-vitro experiments have shown that low-frequency skin impedance may be manipulated not only by iontophoresis but also by varying the ionic strength of the background electrolyte bathing the tissue

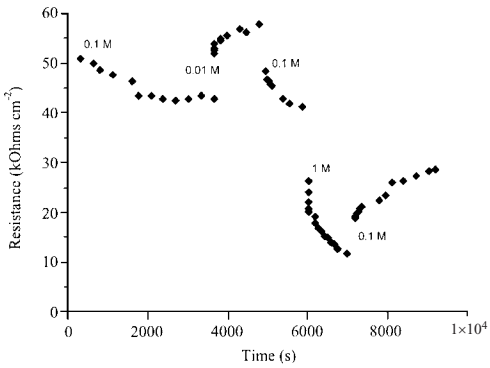

Figure 6 Response of skin resistance, as a function of time, to sequential changes in the ionic strength of the bathing medium on either side of the skin (in-vitro, hairless mouse) (redrawn from Oh et al 1993).

whereby, once again, skin resistance may be changed by altering the level of ions available to carry charge within the membrane (Oh et al 1993) (Figure 6).

The published in-vivo results on the effects of iontophoresis on low-frequency skin impedance are summarized in Table 1. The key observations may be highlighted as follows: increasing the current density applied and increasing the duration of current application elicit greater decreases in impedance, which require concomitantly longer periods of recovery (Kalia & Guy 1995) (Figure 7). Interestingly, application of 0.3 mA cm^{-2} for 5 min reduced low-frequency skin impedance to the same extent as 0.1 mA cm^{-2} for 15 min, that is, when the same total charge was passed across the skin (Kalia et al 1996a). The effect of ionic strength on the iontophoresis-induced decrease and subsequent recovery of skin resistance is less clear-cut. In earlier work, a 10-fold increase in the electrolyte concentration used resulted in a greater change in skin resistance upon iontophoresis and a longer recovery time (Kalia & Guy 1995) (Figure 7). However, in more recent data (Curdy et al 2000), in which different electrolytes (NaCl, KCl, $CaCl_2$, $MgCl_2$) at the same concentration (but different ionic strength) were examined, no significant changes in the decrease and recovery of low-frequency impedance were observed. Equally, the nature of the principal cation present in the electrolyte solution did not influence the response of the skin's electrical properties to iontophoretic current passage (Curdy et al 2000) (Figure 8). The latter data contrast somewhat with a less detailed earlier study (Kalia & Guy 1995), in which NaCl and $CaCl_2$ were compared and it was found that impedance recovered more quickly with the latter (Figure 7).

LDF measurements

There is consensus in the literature that the passage of an iontophoretic current can elicit a perceptible, but usually quite tolerable, sensation and, depending on the current and duration of application, a transient erythema beneath the electrode sites (Ledger 1992). The extent of this local irritation has been followed by LDF after application of the iontophoretic protocol. Different publications using this procedure report levels of increased (relative) skin blood flow to different extents, sometimes with more erythema beneath one or other electrode, sometimes with no difference (Préat et al

Table 1 Impedance spectroscopy: principal observations reported concerning the influence of different iontophoretic parameters on the decrease and recovery of skin impedance after the current passage.

Iontophoretic parameter	Observation	Reference
Current density ↑	Impedance decrease ↑ +recovery delayed	Oh & Guy 1995; Kalia & Guy 1995; Kalia et al 1996a
Time of application ↑	Impedance decrease ↑ +recovery delayed	Kalia & Guy 1995
Ionic strength ↑ (factor of 10)	Impedance decrease ↑ +recovery delayed	Kalia & Guy 1995
Ionic strength ↑ (factor of 3)	No influence on the decrease +recovery of impedance	Curdy et al 2000
pH of the electrolyte solution	No influence on the decrease +recovery of skin impedance	Curdy et al 2000
Electrode separation	No influence	Kalia & Guy 1995
Electrolyte	No influence	Curdy et al 2000
	Recovery faster using $CaCl_2$ as compared to NaCl	Kalia & Guy 1995

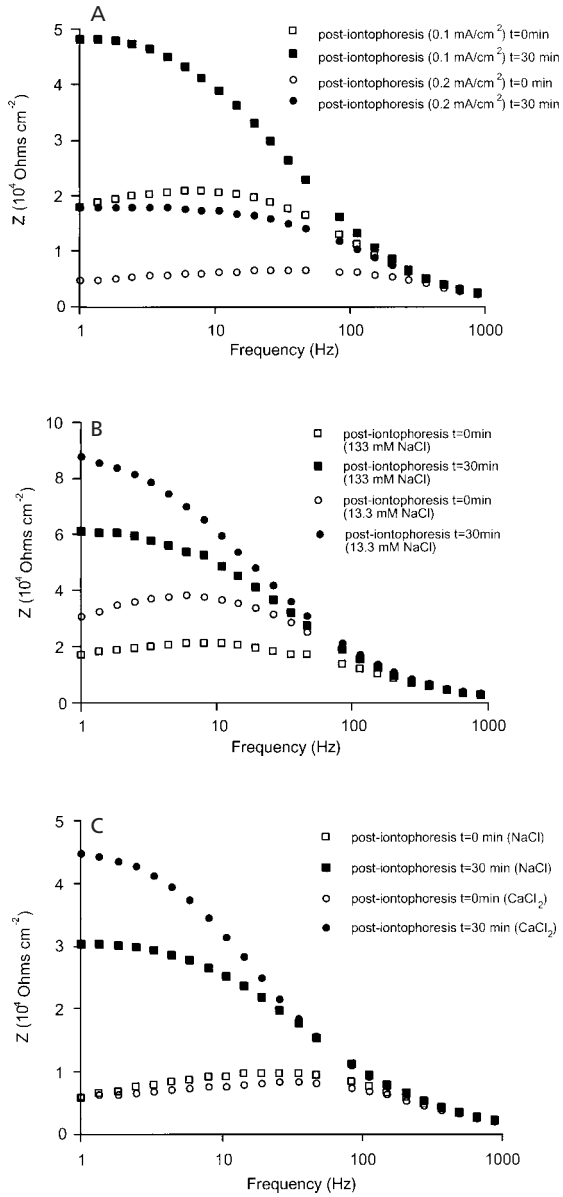

Figure 7 Impedance vs frequency profiles at t = 0 and t = 30 min after iontophoresis of aqueous electrolyte solutions as a function of current density (A), ionic strength (B), and electrolyte composition (C) (redrawn from Kalia & Guy 1995).

1993; Berliner 1997; Brand et al 1997; Van der Geest et al 1996). Direct comparisons are often difficult because the experimental conditions are not always equivalent. For example, both inert metal electrodes (which may cause pH changes that can themselves be irritating; Cullander et al 1993) and reversible Ag/AgCl electrodes have been used. Examples of the observations obtained in this type of investigation are shown in Figures 9 and 10.

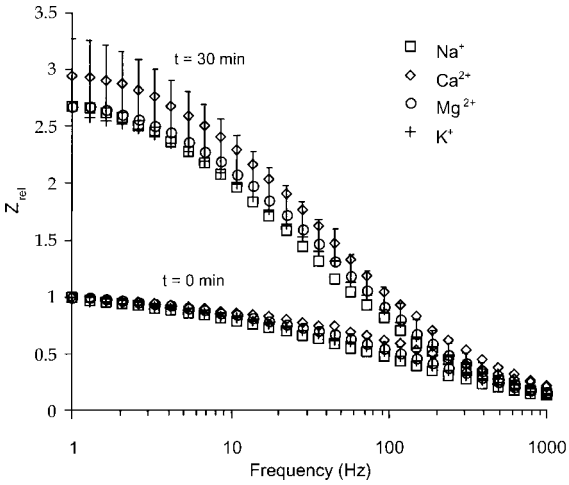

Figure 8 Relative impedance recovery at t = 0 and t = 30 min after 15 min iontophoresis (0.1 mA cm^{-2}) in the presence of either 133 mM NaCl, KCl, $CaCl_2$ or $MgCl_2$ solutions (mean ± s.d., n = 5) (redrawn from Curdy et al 2000).

Discussion

The preponderance of evidence relative to the effects of iontophoresis on human skin in-vivo supports the following conclusions.

(1) On the whole, iontophoresis (at least at modest current densities, < 0.5 mA cm^{-2}) is well tolerated, and the perceptible sensations resulting from current flow are within acceptable limits.

(2) TEWL and reflectance IR spectroscopy measurements indicate that current passage as such does not induce a significant change in skin's basic barrier function. Changes in the rate of TEWL after iontophoresis are accounted for entirely by the hydration induced through contact with the electrolyte used. IR spectroscopy reveals that current passage does not cause significant perturbation of the conformational order of the SC intercellular lipid domains, nor any dramatic effects on protein conformation. These findings are consistent with the perception that iontophoresis amplifies transdermal passage via alternative pathways (the appendages being especially implicated by the literature; Grimnes 1984; Cullander & Guy 1991; Cullander 1992). Biophysical changes at the level of such structures are not presently detectable with typical reflectance IR spectroscopy.

(3) Measurements of skin impedance have proven to be valuable indicators of the rate and extent to which iontophoresis drives ions into the skin, and then how quickly the tissue recovers from the electrical perturbation after application of the current. The sharp

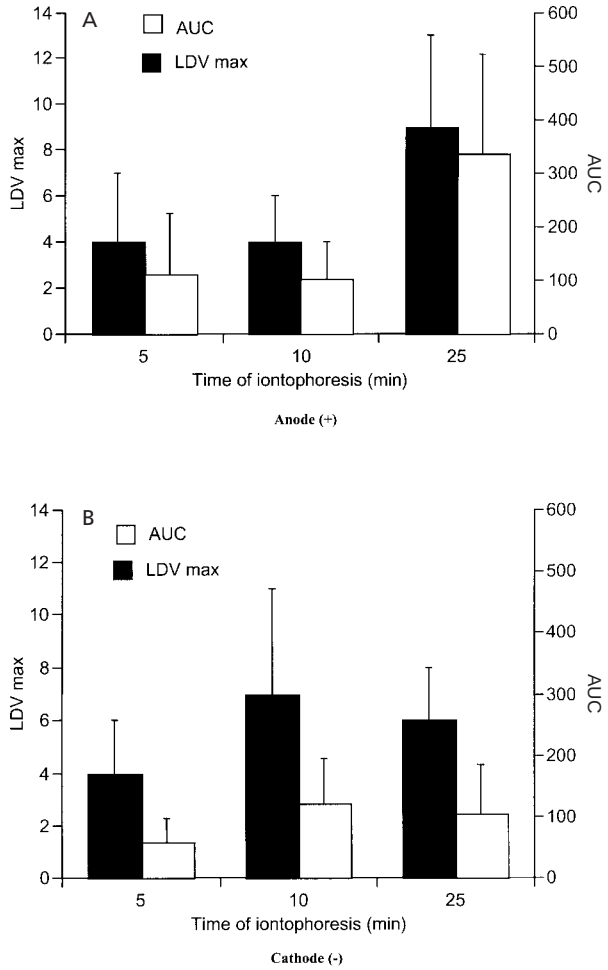

Figure 9 Maximum relative skin blood flow values (LDV_{max}) and areas under the relative skin blood flow vs time curves (AUC) at the anode (A), and the cathode (B), after iontophoresis at 0.5 mA cm^{-2} for 5, 10 and 25 min (mean ± s.d., n = 6 or 7) (adapted from Brand et al 1997).

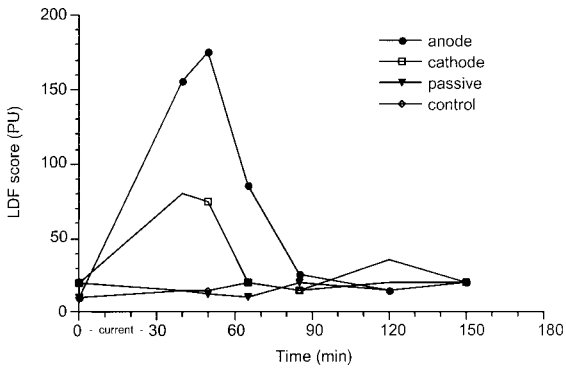

Figure 10 Skin blood flow as a function of time after iontophoresis (0.25 mA cm^{-2} for 30 min) in a human volunteer (redrawn from Van der Geest et al 1996). The site of application was the ventral forearm.

decrease of low-frequency skin impedance upon current application is believed to be consistent with a dramatic increase in the number of ions in the tissue and a concomitant increase in its conductance. Recovery of impedance after iontophoresis is slower than the changes seen when current is applied, presumably because the re-equilibration of ions after current termination can only proceed by passive diffusion. In any case, these changes in electrical properties appear to be reversible and are not in any way reflective of skin damage.

(4) Iontophoresis, in broad terms (at least at current densities used in practice), typically provokes a transient erythema, the intensity and duration of which, as measured by LDF, appear to be related to the intensity and duration of current passage. The effect amounts to a simple irritation, which usually resolves quite quickly. No evidence for current-induced sensitization has been found.

Conclusions

Overall, at the level of the in-vivo response of human skin to iontophoresis, the outlook is relatively promising, in that no dramatic effects have been observed. However, not all experiments reported have been carried out under strictly comparable conditions, which means that interpolation between different datasets must be performed with caution, and more importantly, the published literature concerns, almost exclusively, acute measurements of the effects of iontophoresis on human skin in-vivo. Publication of clinical results from chronic wear applications, as is now beginning to appear (e.g. Tamada et al 1999), is crucial to allay these concerns and to advance the future application of this remarkable skin permeation enhancement strategy.

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