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Acute effects of iontophoresis on human skin in vivo: cutaneous blood flow and transepidermal water loss measurements

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Abstract

The objective of this study was to quantify the acute effects of iontophoretic current passage on human skin in vivo. Specifically, local skin blood flow (SBF) and transepidermal water loss (TEWL) have been measured at the sites of electrode application before and subsequent to iontophoresis at current levels which are generally considered to be 'reasonable'. Infrared spectra were also recorded at the same skin sites using the attenuated total reflectance technique (ATR-IR). The current levels administered were up to 0.5 mA/cm^2 for a maximum of 25 min. It was found that current application for only 5 min was sufficient to cause a significant increase in SBF. Longer periods of current flow induced greater changes in SBF, the elevated level of which persisted for longer times after the termination of iontophoresis. Typically, SBF increased more beneath the anode than beneath the cathode, although visually the degree of irritation was sometimes difficult to distinguish. All subjects were able to feel the application of current, the majority registering greater discomfort at the anode. Apart from the occlusive effect of the electrode chamber solutions, iontophoresis elicited no significant change in TEWL relative to the no-current controls. Similarly, ATR-IR detected no major changes in the spectroscopic profile of the outer stratum corneum. Only relatively minor alterations in protein conformational distribution were observed. In summary, the acute effects of iontophoresis on human skin in vivo are quite moderate. The most significant effect is the rather consistent induction of an erythematous response, the persistence of which depends upon the quantity of charge and the absolute level of current delivered. © 1997 Elsevier Science B.V.

Keywords: Iontophoresis; Skin barrier function; Transdermal drug delivery

1. Introduction

Recent interest in the use of iontophoresis to enhance the transdermal delivery of diverse drugs has been considerable [1]. The attraction of the approach centers around the ability of electrotransport to render skin-permeable some compounds which have essentially no passive flux, and the ability to sensitively control drug input by modulation of the applied current. As a result, the technique has been applied to improving the delivery of both relatively conventional drugs (e.g. lidocaine [2,3]) and that of more complicated peptides (e.g. LHRH analogs [4–9], calcitonin [10,11]), and small proteins (e.g. insulin [12]).

There is, however, a significant literature database which deals with the effects of iontophoresis on the skin, specifically the erythema and mild discomfort induced by current passage [13,14]. It is well-recognized

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that currents, which are typically used in iontophoresis (i.e. a few tenths of a milliampere of skin), are 'felt' by the majority of subjects and often cause visual reddening of the skin beneath the sites of electrode application.

To date, the objective measurement of the skin's response to current passage has been only briefly considered [15]. Nevertheless, it is clear that both the acute and chronic impact of current flow on the cutaneous barrier will need to be fully characterized for any iontophoretic system to be approved for marketing by the regulating authorities. While the electrical properties of the tissue post-iontophoresis can be objectively assessed using impedance spectroscopy [16,17], other alterations in barrier function and evoked response require other bioengineering and biophysical techniques.

In this paper, therefore, we describe evaluation of the acute effects of iontophoresis on human skin in vivo using three distinct approaches: laser Doppler velocimetry (LDV) to measure skin blood flow (SBF) [18], i.e. to quantify the erythematous reaction to current passage; determination of transepidermal water loss (TEWL) to gauge the effect of iontophoresis on the skin's most fundamental and important barrier function [19]; and reflectance infrared spectroscopy (ATR-IR) which can report, at the molecular level, on the status of the stratum corneum, the skin's least permeable and outermost layer [20].

2. Methods

2.1. Subjects

The human volunteers were healthy, young adults (aged 30 ± 5 years), with no history of dermatological disease. The subjects were taking no medication whatsoever during the study. All volunteers were required to read and sign an informed consent document which had been approved by the UCSF Committee on Human Research.

2.2. Iontophoresis

The electrode chambers were simple glass cylinders (typically of area 1 cm^2 and containing approximately 0.5 ml of 0.9% sodium chloride (pH 6.2 ± 0.1)) which were fixed firmly to the ventral forearm surface using silicone grease. The anodc and cathode chambers were 6–8 cm apart. A third 'control' chamber was positioned between the electrode compartments. No current was ever delivered to the skin via this third chamber, which served, therefore, as the untreated, control site. The electrodes were silver–silver chloride which were prepared in the usual way [21] by plating Ag wire (99.4%, Aldrich Chemical, Milwaukee, WI) electrochemically with AgCl. A current was delivered to the electrodes using a commercially available iontophoretic power supply (Phoresor® Iomed, Salt Lake City, UT). The iontophoretic variables examined in the study were as follows:

(1) A constant current of 0.5 mA/cm^2 was delivered for different application times, 5, 10, and 25 min (corresponding to total charge deliveries of 2.5, 5 and 12.5 $mA \cdot cm^{-2} \cdot min$, respectively).

(2) A constant amount of total charge ($5\ mA \cdot cm^{-2} \cdot min$) was delivered to the skin using three different combinations of current density and application time (0.5 mA/cm^2 for 10 min, 0.25 mA/cm^2 for 20 min and 0.1 mA/cm^2 for 50 min).

(3) The effect of electrode chamber surface area was examined briefly in experiments which compared the delivery of 0.5 mA/cm^2 for 10 min to skin areas of 1 and 7 cm^2 (i.e. the total applied current was increased from 0.5 to 3.5 mA).

2.3. Experimental

All measurements were made in a single, well-ventilated room of reasonably constant temperature and humidity (23 ± 2°C, 50–70% RH). Upon arrival at the experimental site, subjects were required to rest quietly for at least 15 min before commencing the protocol. The electrode and control chamber sites were then delineated on the subjects' forearms and pretreatment SBF, TEWL and ATR-IR data were recorded from each area of skin (see below). The chambers were then fixed to the skin, filled with saline, the electrodes inserted and connected to the Phoreser®, and the appropriate current profile was passed between the anode and cathode. The subjects were unaware of the electrode polarity. During current application, the volunteers were asked to assess subjectively the degree of discomfort felt at the two electrode sites. Following termination of the current, the chambers were removed and the skin sites were dried gently were absorbent tissue. A visual assessment of the degree of reddening at the two electrode sites was made by two investigators, one of whom was 'blinded'. ATR-IR spectra were recorded at the three sites as soon as possible post-iontophoresis (see below). Exactly 15 min after current termination, TEWL was measured at the three positions. When LDV measurements were made, the probe was applied to one site, a recording made then moved to the next site, a recording made, and so on. The SBF measurements were initiated as quickly as possible following the end of current passage, and were continued until the pretreatment value was reached, or for at least 60 min.

Table 1
Sites of greatest perceived sensation[a], and of maximum reddening[b] immediately post-iontophoresis, in seven subjects receiving 0.5 mA/cm^2 for 5, 10 or 25 min

Subject	Time of iontophoresis					
	5 min		10 min		25 min	
	Sensation	Redness	Sensation	Redness	Sensation	Redness
1	Cathode	Cathode	Equal	Anode	Anode	Anode
2	Anode	Cathode	—[c]	—[c]	Equal	Anode
3	Anode	Cathode	Equal	Cathode	Equal	Equal
4	Anode	Cathode	Anode	Anode	Equal	Anode
5	Anode	Cathode	Anode	Cathode	Anode	Anode
6	Equal	Cathode	Anode	Equal	Anode	Equal
7	Cathode	Cathode	Anode	Equal	Anode	Equal

[a] Subjective response from the volunteers.
[b] Subjective observation of two investigators (one of whom was 'blinded') for each volunteer.
[c] Not recorded.

2.4. Laser Doppler velocimetry

SBF was measured using a laser Doppler flowmeter (Periflux Pf2, Perimed KB, Sweden) and was quantified as a signal proportional to the product of the number of red blood cells and their velocity. The approach and its theoretical basis have been described in detail elsewhere [22]. For the purpose of comparison, all results were expressed as the ratio of SBF post-iontophoresis to the pretreatment value. As multiple measurements were made at each site, SBF was determined each time over a period of 1 min and the average value was assessed.

2.5. Transepidermal water loss

TEWL was determined using an unventilated evaporimeter (EP1C, Servomed AB, Sweden). As for SBF, TEWL was followed over a period of at least a min until the value had stabilized. The results obtained were the absolute values registered by the evaporimeter.

2.6. Reflectance infrared spectroscopy

Spectra were recorded using a Nicolet 520 spectrophotometer (Madison, WI) equipped with a liquid N_2-cooled mercury-cadmium-telluride detector and a 45° zinc selenide internal reflectance accessory, as previously described [23]. The FT-IR spectra (64 scans at 2 cm^{-1} resolution) were taken from the electrode chamber and control sites, prior to and then immediately following iontophoresis. Skin surrounding the area of interest (i.e. anode, then cathode, then control) was screened to ensure that information only from the treated tissue was obtained. Attention was directed particularly at two regions of the IR spectrum. First, the methylene group symmetric and antisymmetric stretching vibrations near 2850 and 2920 cm^{-1} were examined, following improvement of the spectral resolution to 0.1 cm^{-1} using a center-of-gravity algorithm [24]. The frequencies of the $-CH_2-$ vibrations (ν_{CH2}) report on the conformational disorder of the alkyl hydrocarbon chains of the intercellular lipids of the stratum corneum [20], and have been shown to correlate, for example, with the skin's permeability barrier to water transport [25] (i.e. as ν_{CH2} increases, conformational disorder of the lipid chains increases, and the permeability is greater). Second, the amide I absorbance band ($\sim 1700-1620$ cm^{-1}) contains information about the relative distribution of protein (i.e. predominantly keratin) conformations present in the examined sample. The second derivative of the IR spectra was taken, following baseline correction and subtraction of increased water absorbance (due to hydration), and the relative contributions of various conformations (α-helix, β-sheet, β-strand, turns and random coils) was assessed as previously described [26].

3. Results and discussion

At the highest current density employed (0.5 mA/cm^2), which is that typically quoted as being the upper limit for iontophoresis [14], all subjects experienced mild discomfort (including 'tingling', 'prickly feeling', 'slight stinging sensation') at the electrode chamber and all demonstrated an erythematous response (but without swelling or edema). However, the location of most perceptible discomfort and reddening depended upon the time for which the current had been applied (Table 1). With only a couple of exceptions, most discomfort was perceived either at the anode or equally at both sites. The volunteers all felt, in particular, the initiation of current flow, after which the perceived level of

discomfort subsided. For the 25 min application, the more intense erythema was also associated primarily with the anode. However, at shorter times, it seemed that the cathodal site was more reddened. The significance of this latter observation is not clear at this time, since current application for only 5 min may have little or no clinical utility. At longer times, the data are consistent with that which has been most frequently reported in the literature [27]: that the anode is the electrode at which the most noticeable side-effects of iontophoresis occur. As shown by the SBF time-course data to be discussed below, there is variability in the measurement of erythema, such that visual perception and instrument recordings agree sometimes only qualitatively rather than quantitatively. There were no detectable changes in SBF recorded at the control sites.

The time-course of the resolution of elevated SBF post-iontophoresis is shown, as a function of the different current protocols used, in Figs. 1, 2 and 3. A general observation is that the decay of SBF to the pre-treatment level followed different patterns at the anode and cathode sites (panels (a) in Figs. 1–3, compared with panels (b)). While SBF at the anode remained somewhat constant after termination of current passage before falling to the control level, that at the cathode

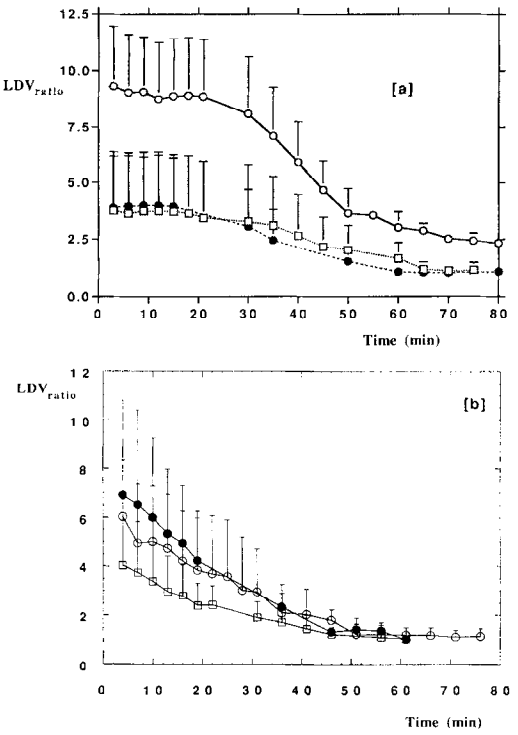

Fig. 1. Relative SBF (LDV_{ratio} = LDV value at time, t following termination of iontophoresis divided by LDV value measured at the same site prior to current passage) as a function of time post-iontophoresis. Panel (a), anode; panel (b), cathode. Treatments: 0.5 mA/cm^2 for 5 min (□), 10 min (●), and 25 min (○). Each point represents the mean ± S.D. for six subjects. Electrode chambers were 1 cm^2 in area.

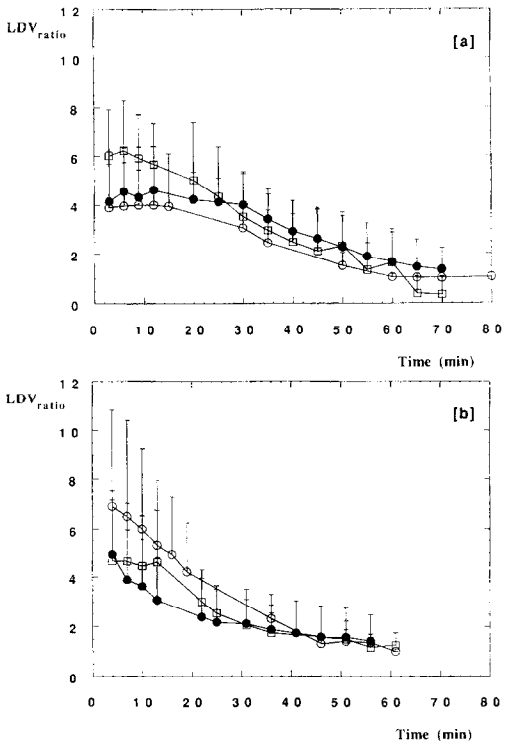

Fig. 2. Relative SBF (LDV_{ratio} defined in legend to Fig. 1) as a function of time post-iontophoresis. Panel (a), anode; panel (b), cathode. Treatments: 0.1 mA/cm^2 for 50 min (□), 0.25 mA/cm^2 for 20 min (●), 0.5 mA/cm^2 for 10 min (○). Each point represents the mean ± S.D. for six subjects. Electrode chambers were 1 cm^2 in area.

decayed monotonically immediately post-iontophoresis. In Table 2, three characteristic parameters, which describe the curves in Figs. 1–3, are presented [28]: (i) the maximum relative SBF value observed (LDV_{max}); (ii) the area under the relative SBF versus time curve (AUC); and (iii) the time required for relative SBF to decay to 75% of its maximum value ($t_{75\%}$).

Fig. 1 shows the decrease of relative SBF as a function of time following iontophoresis at 0.5 mA/cm^2 for 5, 10 and 25 min. At the anode (panel (a)), current passage for 25 min caused a significantly greater (as assessed by ANOVA, $\alpha < 0.05$) elevation in SBF (as measured by LDV_{max} and AUC) than for 5 or 10 min. Resolution of SBF, as determined by $t_{75\%}$, however, was not a function of current application time (Table 2). At the cathode (Fig. 1, panel (b)), on the other hand, relative SBF was affected similarly by 0.5 mA/cm^2 at all treatment times (Table 2).

The effects on SBF following the delivery of a total amount of charge of 5 mA·cm^{-2}·min were independent of the combination of current density and times used (Fig. 2, Table 2), at both the anode and cathode. Fig. 3 demonstrates that the use of a large surface area for current passage did not, in this single example examined, provoke a statistically significant difference in the pattern of relative SBF response post-iontophoresis (ANOVA, $\alpha > 0.05$).

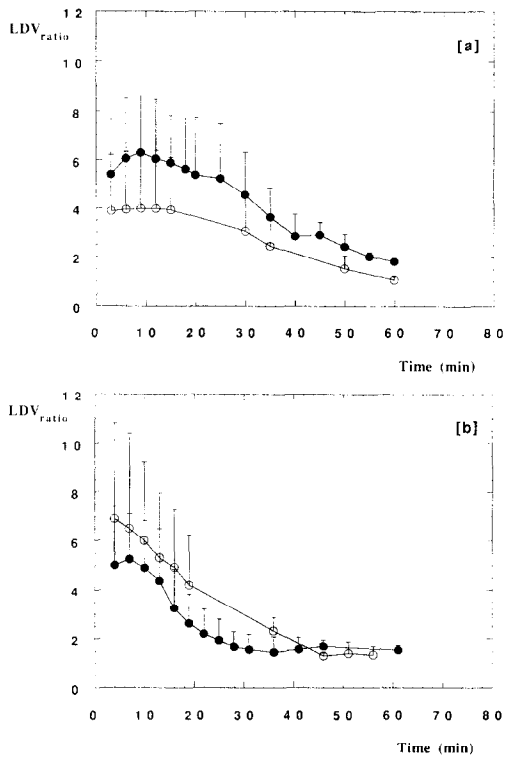

Fig. 3. Relative SBF (LDV_{ratio} defined in legend to Fig. 1) as a function of time post-iontophoresis. Panel (a), anode; panel (b), cathode. The treatment was 0.5 mA/cm^2 for 10 min applied to electrodes positioned in chambers of area either 1 cm^2 (○) or 7 cm^2 (●). Each data point represents the mean ± S.D. for six subjects.

The effect of iontophoresis (0.5 mA/cm^2 for 5, 10 and 25 min) on TEWL is shown in Fig. 4. It was found that, after current passage, TEWL was elevated incrementally with the time of application. However, the 'control' experiment, in which skin was exposed to electrolyte solution for the same period of time but without current passage, gave identical results to those seen beneath the active electrode sites. Thus, it can be concluded that, with the current protocols used here, iontophoresis is not altering the skin's barrier function to water permeability any differently than the simple

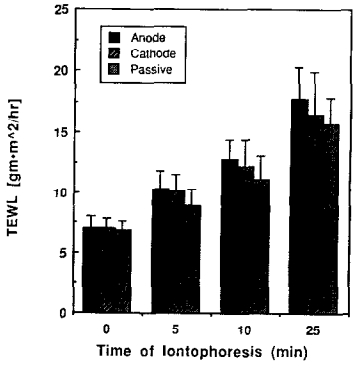

Fig. 4. TEWL measured prior to iontophoresis (plotted at time = 0) and 15 min following either 5, 10 or 25 min of current passage at 0.5 mA/cm^2. Results for the skin sites below the anode, cathode and no-current control chambers are shown for each treatment time. Each data point represents the mean ± S.D. for seven subjects.

increase in hydration which results from passive exposure to an aqueous solution.

Finally, ATR-IR was used to determine whether iontophoresis at 0.5 mA/cm^2 for 5, 10 or 25 min induced any significant changes in stratum corneum lipid and/or protein conformation. Examination of the $-CH_2-$ symmetric and antisymmetric stretching vibrations revealed maximal increases in frequency of approximately 0.5–1 cm^{-1} at anodal and cathodal, and passive control sites (data not shown). No significant differences with respect to time of treatment, or anode versus cathode versus passive control were found. Similarly, unlike the effects of certain penetration enhancers (e.g. oleic acid, organic sulfoxides) [29,30], iontophoresis produced little or no ATR-IR detectable changes in the distribution of different protein conformations, as assessed from the effects of current passage on the second derivative of the amide I absorbance band (data not shown). It must be said, however, that ATR-IR, as used in these measurements, probes only the very superficial layers (i.e. the outermost one or two) of the skin's permeability barrier. Effects of current passage may have occurred deeper into the stratum corneum,

Table 2
Effects of iontophoresis on skin blood flow (data represent the mean ± S.D. for $n = 6$ or 7)

	Treatment			Anode (+)			Cathode (−)		
	Area (cm^2)	mA/cm^2	Time (min)	LDV_{max}	AUC	$t_{75\%}$	LDV_{max}	AUC	$t_{75\%}$
A	1	0.5	5	4 ± 3	111 ± 114[a]	29 ± 4	4 ± 2	59 ± 40	13 ± 4
B	1	0.5	10	4 ± 2	101 ± 69[a]	26 ± 11[b]	7 ± 4	122 ± 73	7 ± 3[b]
C	1	0.5	25	9 ± 4	335 ± 186[a,c]	33 ± 6[b]	6 ± 2	106 ± 78[c]	12 ± 7[b]
D	1	0.1	50	6 ± 2	157 ± 84	15 ± 5	5 ± 2	87 ± 47	11 ± 6
E	1	0.25	20	5 ± 1	139 ± 66	27 ± 10	4 ± 2	70 ± 68	11 ± 15
F	7	0.5	10	7 ± 2	214 ± 94	37 ± 10	5 ± 2	91 ± 58	17 ± 5

[a] The AUC for treatment C is significantly greater than those for treatments A and B.
[b] $t_{75\%}$ at the anode was significantly greater than that at the cathode.
[c] The AUC at the anode is significantly greater than that at the cathode.

but these would have been 'invisible' to the spectroscopic technique as employed here. Tape-stripping would represent one approach by which the deeper layers may be probed [31] although, because of the time involved in removing the skin 'strips', rapidly reversible effects may still be missed.

Overall, the results of this investigation demonstrate that the acute effects of iontophoresis on skin's physical barrier function are relatively modest. Certainly, current passage provokes local, reversible erythema, but it does not appear to significantly interfere with the barrier's principal role in preventing water loss. From other experiments performed in our laboratory and elsewhere, it appears that the skin's electrical impedance is a much more sensitive, and mechanistically informative, parameter with which to characterize the effects of iontophoresis on the skin's fundamental properties, and its ability to recover from the provocation of current flow [16,17].

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