The Effect of Saline Iontophoresis on Skin Integrity in Human Volunteers

I. Methodology and Reproducibility

ETIENNE CAMEL, MICHAEL O'CONNELL,* BURT SAGE,* MICHAEL GROSS,† AND HOWARD MAIBACH

Department of Dermatology, University of California, San Francisco, California 94143-0989, *Becton Dickinson Research Center, Research Triangle Park, North Carolina; and †Becton Dickinson Transdermal Systems, Fairlawn, New Jersey

Received May 16, 1995; accepted April 12, 1996

This study, conducted in 36 human volunteers, was an evaluation of the effects of saline iontophoresis on skin temperature, irritation, and barrier function. The major objectives were to assess the effects of low-level ionic currents, to validate the proposed methodology of assessment, and to establish reproducibility in repeated saline iontophoresis applications. This was the first of a multistage study designed to assess the safety of 24-hr saline iontophoresis episodes at selected currents and current densities. Since an iontophoresis patch challenges the skin barrier both by occluding the skin surface and by passing ionic current through the skin, the experimental protocol was designed to permit measurement of the contribution of each of these processes to the overall response. In this first stage we investigated the effect of 10 min of current delivery, at 0.1 mA/cm² on a 1-cm² area patch and 0.2 mA/cm² on a 6.5-cm² area patch compared to unpowered control patches. Twelve subjects were tested under each condition on two separate occasions to examine reproducibility of the response variable measurements. A further 12 subjects were tested once under the 0.2 mA/cm², 6.5-cm² condition. Skin irritation was evaluated via repeated measurements of transepidermal water loss, capacitance, skin temperature, skin color, and a visual scoring system, before the iontophoresis episode and after patch removal. No damage to skin barrier function in terms of skin-water loss or skin-water content was detected. Slight, subclinical, short-lasting erythema was observed for both conditions. Assessment of correlation coefficients showed highly statistically significant indications of reproducibility for all five response variables measured. The experimental design, in combination with a repeated measures analysis, provided clear separation of the occlusion and ionic current components of the iontophoretic patch challenge. Further, the repeated measures analysis gave a highly sensitive assessment of skin irritation and resolution after patch removal. We conclude that the experimental methodology is appropriate for assessing possible changes in skin integrity resulting from saline iontophoresis under similar operating conditions for longer durations and for other skin challenges from which a subclinical response is expected.

1. INTRODUCTION

Over the past decade considerable attention has been given to the development of new techniques for controlled transdermal drug delivery. Percutaneous absorption provides well-described attractive advantages, but the diffusional barrier of the stratum corneum has often limited its use and has motivated research on chemical enhancers (Goodman and Barry, 1989), ultrasound (Kost et al., 1989), and iontophoresis (Sage and Riviere, 1992; Singh and Roberts, 1989). Historical interest in iontophoresic drug delivery dates back to the work of Leduc (1900) and Turrell (1921). Recent technical advances including electronics miniaturization and new peptide therapeutics have encouraged consideration of iontophoresis as a drug delivery method. There are, however, important medical issues concerning the long-term use of iontophoresis that have not been extensively investigated. Of particular interest in this study are the effects of electrode size, current and current density magnitude, iontophoresis duration, and electrolyte concentration on human skin integrity.

Iontophoresis opens the possibility for long-term transdermal delivery of compounds that cannot penetrate the skin by passive diffusion. Many in vitro studies have employed operating conditions that are unsuitable for human therapy with respect to cutaneous tolerance. Burns and discomfort have been reported following iontophoresis (Holzle and Alberti, 1987; Zeltzer et al., 1991) and have constituted the main objection against the long-term application of the method. These undesirable responses may be due to faulty design or application of the electrodes and to the use of excessively high current and/or voltage (Molitor and Fernandes, 1939). Recent animal studies have focused on the effect of electrical current on skin structure using appropriate op-
erating conditions. Cho and Kitamura (1988) described edema and significant histological changes on the structure of the guinea pig tympanic membrane. Monteiro-Riviere (1990) observed erythema and edema on pig skin associated with microscopic changes that did not seem to be, according to the authors, of a practical toxicological significance since they resolved within hours or days. In addition to these few well-described studies on skin structure, reports on the effect of electrical stimulation on skin function are not numerous and, as stated by Ledger (1992) in a review of iontophoresis, "the literature concerning clinical experiments tends to be anecdotal rather than systematic."

In a review of the literature on the dermal effects of iontophoresis, we were unable to discover a single report of a study designed to evaluate the changes in the integrity of the barrier function of skin under electrical conditions appropriate for iontophoretic drug delivery. Indeed, in those reports which were located, comments on skin effects were secondary to the main theme of the report—the therapeutic or diagnostic effects of delivering specific chemical agents by iontophoresis.

During iontophoresis, the skin barrier function is challenged by three processes: the accumulation of the chemical agent in the skin, occlusion of the skin by the iontophoresis patch, and the passage of physiological ions such as sodium and chloride through the skin. This last process is not insignificant since, during iontophoresis, the vast majority of the electrical current is carried by these physiological ions, not the drug ion.

The objective of this study was to measure the effects of passing ionic current through the skin. For this reason, physiological saline was the electrolyte solution placed in the patch. A further objective was to separate the effects of occlusion and ionic current flow and the experimental protocol was designed so that the separate effects of each process on the measured responses could be determined. A final objective was to conduct a short episode of iontophoresis similar to episodes used to treat hyperhidrosis in order to provide a range of values for each of the measured responses. This allowed reasonable assessment of response reproducibility and current effects on the responses.

We conducted iontophoresis for 10 min using saline solutions to evaluate methods of assessing skin integrity before testing longer duration episodes and pharmaceutical compounds. We investigated the effect of two iontophoresis treatment conditions on skin integrity and measured several responses, namely transepidermal water loss (TEWL), skin capacitance, skin temperature, skin color, and primary skin irritation via the visual scoring system of Draize et al. (1944).

2. MATERIAL AND METHODS

2.1. Iontophoresis Equipment

2.1.1. Iontophoresis power source. The battery-powered iontophoresis power source used in this study was designed by Becton Dickinson to provide a user-selectable constant current in the range 0.1 to 5.0 mA. The power source further provided patient protection circuits which automatically stopped the current in the event of an inadvertent open circuit or short circuit or an excessive rate of voltage change.

2.1.2. Iontophoresis patches. The iontophoresis patch construction is shown in Fig. 1. The patches were filled with sterile isotonic USP saline solution just prior to use. An important feature of the patch design is the use of silver as the anode material and silver/silver chloride as the cathode material. This choice of materials provides stable formulation pH over the duration of the iontophoresis episode (Sage and Riviere, 1992).

2.2. Operating Conditions

Two operating conditions were studied and these are described below. We refer to these as the low current condition (group A) and high current condition (group B). Iontophoresis episodes were 10 min in duration. The currents applied in the two groups were chosen to be quite different in order to provide a range of values for each of the measured responses. This allowed reasonable assessment of response reproducibility and current effects on the responses.

<table>
<thead>
<tr>
<th>Group</th>
<th>Current (mA)</th>
<th>Anode area (cm²)</th>
<th>Cathode area (cm²)</th>
<th>Anode current density (μA/cm²)</th>
<th>Cathode current density (μA/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.1</td>
<td>1</td>
<td>2.88</td>
<td>100</td>
<td>.35</td>
</tr>
<tr>
<td>B</td>
<td>1.25</td>
<td>6.25</td>
<td>12.2</td>
<td>200</td>
<td>90</td>
</tr>
</tbody>
</table>

2.3. Study Population and Design

A total of 60 volunteers, all either Caucasian or Hispanic, were recruited in accordance with specific and well-established inclusion criteria. Of these, 36 were randomly selected to participate in the study and gave written consent. The study was approved by the University of California San Francisco Committee on Human Research. The study group consisted of 18 males and 18 females. Six females and six males where assigned to group A and were tested under the conditions described above. All of these individuals returned 1 week later to be retested under the same conditions. Twelve females and 12 males were assigned to group B. Of these, 6 females and 6 males were randomly selected to return a week later for the same test.
2.4. Methods

Environmental conditions were monitored hourly: room temperature was 20–26°C and relative humidity 46–58%. These observed ranges were small and the conditions were found to be unrelated to the measured responses. Transepidermal water loss (TEWL) and skin capacitance provided an objective evaluation of changes in skin barrier function. TEWL is a particularly well-accepted method for assessing irritation potential of topically applied materials (Idson, 1978; Van Der Valk et al., 1984). Irritation tends to reduce the efficiency of the stratum corneum barrier function and results in an increase in TEWL. This is sometimes associated with a decrease in skin water content (Wilhelm et al., 1989; Cua et al., 1990). Hence, measurement of skin capacitance or skin hydration (Tagami et al., 1980) may also be used to assess irritation (Thiele and Malten, 1973; Serban et al., 1981).

TEWL (mg/m²/hr) was measured with an Evaporimeter (EpI Servo Med, Stockholm, Sweden) using the method of vapor pressure gradient calculation previously described by Nilsson (1977). The probe was held on the skin until stable TEWL values were established (30 sec) and the average of two readings obtained during the next 30 sec was recorded. Skin capacitance (device internal units, i.u.) was measured in duplicate using a Comeometer SM 820 PC (Courage & Khazaka, Cologne, F.R.G.) previously described by Van Neste (1991) and developed to measure the water content of the stratum corneum. Skin temperature, an important factor effecting the rate of TEWL of normal skin, was measured using an infrared thermometer (OS 612, Omega Engineering Inc., Stamford, CT) which allows fast digital readings.

Skin color was evaluated with a Minolta Chromameter CR-300 (Minolta Corp., Ramsey, NJ) using reflectance techniques. Color was defined in accordance with the standardized tristimulus system recommended by CIE (Commission Internationale de l’Eclairage). Estimates of changes in redness intensity were made from the “a*” scale (100 = red only, -100 = green only; Wilhelm et al., 1989). Primary skin irritation was evaluated by an individual blind to operating conditions. The visual scoring system described by Draize et al. (1944) was used to grade erythema: 1 = very slight erythema, barely perceptible, 2 = well-defined erythema, 3 = moderate to severe erythema, 4 = severe erythema (beet redness) to slight eschar formation (injuries in depth). For edema, 1 = very slight edema (barely perceptible), 2 = slight edema (edges of area well-defined by definite raising), 3 = moderate edema (area raised approximately 1 mm), and 4 = severe edema (raised more than 1 mm and extending beyond area of exposure). Photographs were taken with a dental eye camera equipped with an auto ringflash (Yashica, Japan).

Voltage and current were monitored using a Rustruk Ranger data acquisition system (Gulton/Rustruk, East Greenwich, RI) and recorded on a personal computer for data analysis. Skin impedance was calculated as the ratio of voltage to current.

2.5. Procedure

Volunteers stayed in the room for 20 min, both arms exposed prior to the recording of any measurements. Baseline measurements were taken on the right and left volar forearms on delimited sites corresponding to the cathode and the anode of the patch. Application sites were located 3 in. from the wrist. The central anode of the patches, which acted as the reservoir, was made of an inert polymeric material and was filled with saline solution (sodium chloride, 0.9%; Abbott Laboratories, North Chicago, IL). One patch was applied on each of the delimited sites and, in accordance with a previously established randomization schedule, one was connected to a power source. Patches were wrapped with elastic bandages (ACE brand woven fabric, nonocclusive) to ensure that the electrodes did not move.
The unactivated patch acted as a control to evaluate the separate effects of occlusion and iontophoresis on the skin irritation measurements. Placement of the active patch was balanced for arm within sex by treatment group. Subjects were blind regarding the active and control patch placement. Current was delivered for 10 min. After patch removal application sites were gently wiped with gauze to remove any excess solution. Skin temperature and color were then immediately measured and visual score was assessed at the anode and cathode site of each patch, active and control. Skin temperature measurements were repeated 30 min and 1 hr after patch removal and visual scores and skin color measurements were repeated 30 min and 1 and 2 hr after patch removal. Transepidermal water loss and electrical capacitance were measured 15 min and 1 hr after patch removal at both the anode and cathode sites.

2.6. Statistical Analysis

The primary planned comparisons of the study involved assessment of the extent of skin reactions to iontophoresis in terms of the measured responses, and in particular to determine the onset and resolution of these iontophoresis effects, at both the anode and cathode sites. Response variables for this analysis were formed by subtracting the measurement recorded at the control site from that at the corresponding active site. This removed any materials and occlusion effects which may have occurred at both the active and control sites, but to a similar extent. Since each measured response was recorded on each subject at baseline and several times after the iontophoresis episode, a repeated measures analysis was undertaken (Koch et al., 1988) on both the anode and cathode. The repeated measures analysis takes account of correlation between the repeated measurements within individuals.

The first part of the analysis of iontophoresis effects involves an evaluation of the differences in the response variables among the times of observation, including the baseline. Multivariate analysis of variance tests were based on Wilk’s lambda (Mardia et al., 1979). If the overall time effect was found to be significant, and the design factor effects insignificant, contrasts between the baseline response and the responses at each time after the iontophoresis episode were considered using all the data. These contrasts take the form $c_i = (c_{ai} - c_{ai}) - (c_{ci} - c_{ci})$ where $c_{ai}$ is the measured response at the active site at time $t$ and $c_{ci}$ is the measured response at the control site at time $t$. The contrasts, $c_i$, between observation times, define the iontophoresis effects and give a natural decomposition of the observations over time.

The contrasts were also analyzed with respect to the design factors sex, arm, treatment group, and visit. If the overall time effect was significant and at least one of the means in the contrasts analysis was significant, a plot of the contrast means versus time was constructed. This plot shows the overall effect of iontophoresis including its onset and resolution. If an interaction between time and a design factor (sex, arm, group, visit) was significant, the plots of the contrast means were split by levels of the design factor.

A significance level of 0.01 was used for all testing, reporting of differences under Results, and construction of confidence intervals (Miller, 1966). A significant ($p < 0.01$) effect of iontophoresis at any time is indicated by confidence intervals that do not overlap the horizontal line at zero on the graphs.

Reproducibility of the response measurements was assessed via estimation of correlation between responses for the two visits. Correlation coefficient estimates were based on average responses for each subject at each visit in order to avoid induced correlation due to effects of common time after iontophoresis. Correlation coefficients were tested for difference from zero with a Fisher $z$ test.

3. RESULTS

In many cases, there were no significant effects discovered. In these cases, there is no presentation or discussion of results, and the reader may assume an hypothesized effect was insignificant if it is not presented below.

3.1. Effects of Iontophoresis on the Measured Responses: TEWL

There were no significant differences in the measured TEWL between the active and control sites among the times of observation (baseline, 15, and 60 min) at both the anode and cathode of the system.

As shown in Fig. 2, however, a clear rise above baseline for both the active and control anodes was seen 15 min after patch removal, and a clear decrease below baseline for both the active and control cathodes was seen 60 min after patch removal.

3.2. Effects of Iontophoresis on the Measured Responses: Capacitance

The measured capacitance difference between the active and control anodes was found to be elevated at 15 min,
FIG. 4. Capacitance: Occlusion and materials effects after 10 min of iontophoresis. Active and control site means are plotted separately for both the anode and the cathode sites. Intervals around means represent ±1 standard error. The horizontal dotted line shows the baseline value in each case. Simultaneous departures of both active and control means from baseline represent occlusion/materials effects.

compared to baseline and the 60-min measurement. However, no significant differences between the active and control cathode were found. Upon further investigation, this increased capacitance at the anode was specific to males (Fig. 3) and disappeared at 60 min. The mean capacitance change at the anode was lower on the right arm (−3.7) than the left arm (+3.8) over the entire study (p < 0.0001).

When the measured capacitance at the active and control electrodes was compared to baseline, a decrease was seen at 60 min at the anode, and an increase was seen at both 15 and 60 min at the cathode (Fig. 4).

3.3. Effects of Iontophoresis on the Measured Responses: Skin Temperature

The difference in skin temperature between active and control anodes and cathodes was found to be elevated compared to baseline (p < 0.0001) immediately after patch removal and 30 min after patch removal (Fig. 5). The difference was not significant at 60 min.

The mean skin temperature rise on the right arm (0.45°C) was greater than on the left arm (−0.08°C) at the anode over the entire study (p < 0.0001). No such effect was seen at the cathode.

When the measured temperatures at the active and control anodes were compared to baseline (Fig. 6), a significant decrease was found immediately after patch removal. At the cathode, however, an increase was found (Fig. 6). In both cases, these differences were no longer significant 60 min after patch removal.

3.4. Effects of Iontophoresis on the Measured Responses: Skin Color

As measured by the chromameter, skin color compared to baseline was significantly elevated at all measured time points for both the anode and the cathode (p < 0.0001). As is further shown in Fig. 7, the measured color for Group B (1.25 mA over 6.5 cm²) was increased over the measured color for Group A (0.1 mA over 1 cm²), particularly at patch removal and 30 min after patch removal. The color change was in proportion to the current density (100 μA/cm² for Group A and 200 μA/cm² for Group B) at the anode; no such proportionality was observed at the cathode. The measured color was decreased to roughly half the maximum value by 60 min after patch removal.

3.5. Effects of Iontophoresis on the Measured Responses: Visual Score

a. Erythema. Compared to baseline values, the level of erythema was elevated at the anode at all measured time
**FIG. 5.** Effect of iontophoresis on skin temperature at the anode and cathode sites. Values plotted are mean ($s_{t_i} - s_{t_0}$) for each time $t$ after patch removal where $s_{t_i}$ and $s_{t_0}$ are the measured skin temperature at the active and control sites at time $t$, respectively. These differences or contrasts of measurements recorded at time $t$, ($s_{t_i} - s_{t_0}$), with the baseline measurements ($s_{t_0} - s_{t_0}$), define the iontophoresis effect on skin temperature at each time $t$ after patch removal. Confidence intervals (99%) are plotted around each contrast mean. A significant ($p < 0.01$) effect of iontophoresis is indicated for confidence intervals that do not overlap the horizontal zero line.

points for Group B and at the first two measured time points for Group A ($p < 0.0001$) (Fig. 8). At the cathode, as was seen for skin color (see section 3.4), there was no difference between the two groups, and the mean visual score was significantly different from baseline for the first three time points ($p < 0.0001$) (Fig. 9).

Also, at the anode, the visual score was significantly higher on the right arm compared to the left arm immediately after patch removal and 30 min after patch removal (Fig. 8).

**b. Edema.** No edema was observed. In Group B, 2 of the 24 subjects exhibited small papules and vesicles. These were randomly scattered at the anode site of the active patch and resolved quickly (20, 90 min).

### 3.6. Correlation of Responses

Correlation coefficients were calculated between visits for each of the five responses. In every case a highly significant indication of reproducibility within the studied individuals was obtained ($r > 0.75$, $p < 0.0001$). There were no noteworthy correlations between any of the five responses ($r < 0.5$).

### 3.7. Skin Impedance

Impedance was calculated just before current delivery was terminated. The average impedance values were $45,600 \pm 19,200 \, \Omega$ for group A and $5470 \pm 1980 \, \Omega$ for group B. These measurements were recorded only to monitor current delivery.

### 4. DISCUSSION

In the early 1940s, John Draize at the FDA developed dermatological evaluation systems dependent solely on visual and palpatory scoring (Draize et al., 1944). These systems remain the standard. Recently, in searching for more sophisticated assessment of subtle previsible and palpatory changes, skin bioengineering methods have developed (Berardesca et al., 1994; Elsner et al., 1994). In a relative sense, the findings documented with the iontophoretic exposures herein are distinctly less than the findings which would be documented with skin exposures to many surfactants and adhesives (Berardesca et al., 1994; Elsner et al., 1994). In any instance, the measurement techniques employed in this
study permit the facile comparison of any type of ionto-
phoresis system to another drug delivery system (e.g., topi-
cal, transdermal) at a subclinical level without the necessity
of producing clinical lesions.

4.1. Interpretation of Measured Responses: TEWL

The absence of a measurable difference between the active
and control sites indicates that iontophoresis under the condi-
tions studied has no impact on the barrier function of the
skin as measured by TEWL.

The measured increase at both the active and control anodes (Fig. 2) compared to the active and control cathodes
15 min after patch removal is believed to be due to the compositional differences between the anodes and the cath-
odes. The anode, composed of an aqueous solution of elec-
trolyte, leaves a thin film of solution on the skin after re-
moval. The cathode, composed of a conducting hydrogel,
leaves no such film. The evaporation of the solution film
from the anode skin site results in an apparent water loss
for both active and control.

The depression of TEWL below baseline 60 min after
patch removal at the cathode indicates a drying of the skin
at this site. Given that the same result was measured for
both the active and control cathodes, the effect is a result of
occlusion of the skin by the hydrogel and not a result of
iontophoresis.

4.2. Interpretation of Measured Responses: Capacitance

As noted under Results (section 3.2), an elevation in ca-
pacitance between active and control anodes 15 min after
patch removal was found in males but not in females (Fig.
3). An increase in capacitance is understood to be an increase
in skin hydration. This suggests that there is a small differ-
ence in the ability of male and female forearm skin to retain
water, by either preventing accumulation in the female or
preventing drainage in the male. The clinical significance of
this finding is not known.

Compared to baseline for both the active and control
anodes and active and control cathodes, there are clear
changes in capacitance which are not related to iontophoresis
(Fig. 4). At the anode, capacitance is decreased at 60 min,
and at the cathode the capacitance is elevated at both 15 and
60 min, indicating that the skin under the anode is less
hydrated and the skin under the cathode is more hydrated
at these times.

4.3. Interpretation of Measured Responses: Skin
Temperature

As shown in Fig. 5, there is a small elevation in the skin
temperature at both the active anode and cathode compared

FIG. 6. Skin temperature: Occlusion and materials effects after 10 min of iontophoresis. Active and control site means are plotted separately for
both the anode and the cathode sites. Intervals around means represent ±1 standard error. The horizontal dotted line shows the baseline value in each
case. Simultaneous departures of both active and control means from baseline represent occlusion/materials effects.
FIG. 7. Effect of iontophoresis on Chromameter color (a* units) at the anode and cathode sites. Values plotted are mean ((ch\textsubscript{u} - ch\textsubscript{c}) - (ch\textsubscript{d} - ch\textsubscript{b})) for each time t after patch removal where ch\textsubscript{u} and ch\textsubscript{d} are the measured Chromameter color at the active and control sites at time t, respectively. These differences or contrasts of measurements recorded at time t, (ch\textsubscript{u} - ch\textsubscript{d}), with the baseline measurements (ch\textsubscript{b} - ch\textsubscript{c}), define the iontophoresis effect on the Chromameter color at each time t after patch removal. Confidence intervals (99%) are plotted around each contrast mean. A significant (p < 0.01) effect of iontophoresis is indicated for confidence intervals that do not overlap the horizontal zero line. The anode plot is split by current condition.

to the control anode and cathode. This is an expected finding since the skin is electrically resistive and passing current through the skin results in Joule heating. Given the currents passed through the skin and the measured skin impedances (section 3.8), the electrical power dissipated in the skin is on the order of 5 mW.

At this level of power input, it is unlikely that this Joule heating is the primary cause of the temperature rise. Iontophoresis is known to induce a modest erythema in the skin under the electrode; the results of this study demonstrate this effect as well. The observed erythema is almost certainly due to increased blood flow. The increased blood flow is the most likely cause of the observed temperature rise.

When the active and control electrode sites are compared to baseline (Fig. 6), a small decrease, which returns to baseline, is seen at the anode, and a small increase, which returns to baseline, is seen at the cathode. Given that there is an evaporating film of water at the anode, some degree of evaporative cooling is expected. At the cathode, where there is no evaporative cooling and increased water content, a rise in skin temperature is measured. Since the added water came from the higher temperature water of the body (37°C), it is not surprising that some of this heat is conducted through to the skin surface.

4.4. Interpretation of Measured Responses: Skin Color

It is well known that a clearly discernible reddening of the skin results from iontophoresis (e.g., Ledger, 1992). The results of this study are no different from those of other reports, except that most other studies did not include a passive control to discriminate the components of color due to occlusion and current. The results of this study clearly show that most of the change in color results from the current.

Interestingly, the ability of current to generate color is different at the anode compared to the cathode. At the anode, the degree of color appears to be roughly proportional to current density, while at the cathode there is no measurable difference in spite of a factor of three difference in current density. The reason for the differences in dependence on current density remains to be determined.
FIG. 8. Effect of iontophoresis on visual score at the anode site. Values plotted are mean 
\((v_{s_0} - v_{s_{0-2}} - (v_{s_{0-3}} - v_{s_{0-4}}))\) for each time \(t\) after patch removal split by group and arm, where \(v_{s_0}\) and \(v_{s_t}\) are the measured visual scores at the active and control sites at time \(t\), respectively. These differences or contrasts of measurements recorded at time \(t\), \((v_{s_0} - v_{s_{0-3}})\), with the baseline measurements \((v_{s_{0-3}} - v_{s_{0-4}})\), define the iontophoresis effect on the visual score at each time \(t\) after patch removal. Confidence intervals (99%) are plotted around each contrast mean. A significant \((p < 0.01)\) effect of iontophoresis is indicated for confidence intervals that do not overlap the horizontal zero line.

Given the modest reddening measured, and the reduction measured at 60 min, this response to iontophoresis is clearly subclinical.

4.5. Interpretation of Measured Responses: Visual Score

a. Erythema. Given the correlation between the measured skin color and the visual score for erythema, the interpretation of this response is the same as for skin color, that is, a measurable but subclinical response (see section 4.4).

b. Edema. The fact that no general edema was detected is another indication that the iontophoresis challenge of this study is mild. The low incidence of papules, only rarely seen at the anode of Group B, suggests that the prevalence of small, low impedance (and hence high current) pathways in apparently intact skin is low.

4.6. Interpretation of Measured Responses: Correlation of Responses between Visits

Ideally, a fresh iontophoresis patch could be placed on the same skin location day after day and still be well tolerated. In this study, one group of subjects was treated identically in two treatment episodes separated by 7 days. The objective was to determine if the measured responses of the second treatment were different from the measured responses of the first treatment. No such differences were measured, suggesting that after 7 days the skin had returned, in terms of these measurements, to its naive condition.

There is no intent to imply that it is safe to make repeated applications of iontophoresis on the same skin site. More applications and more frequent applications should be studied.

4.7. Interpretation of Measured Responses: Correlation of Responses

The only correlation between responses was a correlation between the chromameter erythema reading and the visual score for erythema. This was expected given that the chromameter reading used is indicative of a reddening of the skin. However, the chromameter provides only an average reading for the skin site. The visual score can reflect textural information observed at the site. While it is comforting to detect this correlation, there is no suggestion that the chromameter reading could replace the visual score for iontophoresis studies.
FIG. 9. Effect of iontophoresis on visual score at the cathode site. Values plotted are mean $(v_s, - v_{c,0})$ for each time $t$ after patch removal split by group and arm, where $v_s$ and $v_c$ are the measured visual scores at the active and control sites at time $t$, respectively. These differences or contrasts of measurements recorded at time $t$, $(v_s, - v_{c,0})$, with the baseline measurements $(v_s, - v_{c,0})$, define the iontophoresis effect on the visual score at each time $t$ after patch removal. Confidence intervals (99%) are plotted around each contrast mean. A significant $(p < 0.01)$ effect of iontophoresis is indicated for confidence intervals that do not overlap the horizontal zero line.

4.8. Interpretation of Measured Responses: Skin Impedance

The measured skin impedance permits several important conclusions regarding the design of iontophoresis systems. The first conclusion concerns the importance of using constant current power sources. By Faraday’s law, drug delivery is proportional to the current. If constant voltage sources were used, given the observed variability of the impedance, the actual drug delivery could vary by a factor of 3. Second, from the current conducted and the measured impedances, the driving voltages can be calculated: $4.56 \text{ V}$ for Group A and $6.84 \text{ V}$ for Group B. These voltages are very similar despite current levels more than a factor of 10 different, implying that the skin acts like parallel resistors, with a fairly uniform average conductivity per unit area.

The third conclusion relates to the power which is required to conduct these currents. Electrical power is calculated as $P = IR$, where $I$ is the current and $R$ is the resistance. The average power for Group A is 0.00046 W and for Group B is 0.00855 W. Compared to power required for diathermy or phonophoresis (W/cm²), these power requirements are low. Further, it is seen that required power is roughly proportional to the current.

4.9. Overall Interpretation of Measured Responses

While the findings of this study show that under the conditions studied there are minimal effects on the skin, there were clearly measurable effects. These were short-lived color and temperature changes which may be the forerunners of an inflammatory response. Long-term studies, for which this study is preparation, are noticeably absent in the literature and are needed to determine the extent to which the observed erythema and temperature change is a harbinger of inflammation.

A possible weakness in this study relates to the use of different materials in the anode and the cathode of the iontophoresis devices. This resulted in expected small differences in some measured variables which perhaps distract from the main result of skin effects of iontophoresis. A preferred design would use similar anode and cathode materials.

The three main objectives of the study were achieved: effects of low-level ionic current were assessed independently of the effect of occlusion, the experimental methodology was validated, and reproducibility of all measured responses was established. By using an identical control (passive) patch for each episode, and by taking baseline measurements at each site before the patch was positioned, the effects of occlusion were separated from the effects of ion flow. In addition, this design and the associated repeated measures analysis provided a very sensitive measurement of iontophoresis and occlusion effects on skin barrier function. This sensitivity results from repeated measurements on the same subjects and the use of within-subject error for tests across time, essentially avoiding higher between-subject variability for some of the comparisons. As a result, small changes in the measured responses from baseline were able to be detected and their resolution over time was precisely established.

In conclusion, we have validated an experimental procedure for measuring selected properties of the skin which relate to the integrity of the barrier function after treatments which give a subclinical response. This procedure is capable of separating the effects of skin occlusion by the patch and ion flow due to the impressed electric field. Based on these findings, we feel confident in extending these studies of iontophoresis to longer treatment durations and a wider range of operating conditions.

ACKNOWLEDGMENTS

This work was supported by Becton Dickinson Transdermal Systems and we thank W. McArthur and R. Flower for providing assistance with the iontophoretic equipment. In addition, R. Bartel, P. Bloomfield, H. Davis,
REFERENCES


