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An in vivo investigation of the rabbit skin responses to transdermal iontophoresis

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Abstract

To optimize the benefits of transdermal iontophoresis, it is necessary to develop a suitable animal model that would allow for extensive assessments of the biological effects associated with electro-transport. Rabbit skin responses to iontophoresis treatments were evaluated by visual scoring and by non-invasive bioengineering parameters and compared with available human data. In the current density range 0.1–1.0 mA/cm^2 applied for 1 h using 0.9% w/v NaCl and 0.5 mA/cm^2 for up to 4 h, no significant irritation was observed. 2 mA/cm^2 applied through an area of 1 cm^2 for 1 h resulted in slight erythema at both active electrode sites but without significant changes in transepidermal water loss (TEWL) and laser Doppler velocimetry (LDV). A value of 4 mA/cm^2 under similar conditions caused moderate erythema at the anode and cathode with TEWL and LDV being significantly elevated at both sites; 1 mA/cm^2 current applied for 4 h, caused moderate erythema at both anode and cathode; and 1 mA/cm^2 applied for 1 h caused no irritation when the area of exposure was increased from 1 to 4.5 cm^2. When significant irritation and barrier impairment occurred, the erythema was resolved within 24 h with barrier recovery complete 3–5 days post-treatment. Rabbit skin thus shows promise as an acceptable model for iontophoresis experiments.

Keywords: Iontophoresis; Skin; Irritation; erythema; Transepidermal water loss; Laser Doppler velocimetry

1. Introduction

Significant passive diffusion through the skin is limited to small, mainly lipophilic molecules. Physical and chemical techniques have been employed to increase temporarily, the permeability of the skin to applied substances. Iontophoresis is one such method. The technique of iontophoresis involves the facilitated transport of drug molecules under the influence of an applied electric field (Leduc, 1900) which by definition, suggests that the ideal drug candidate for this mode of delivery should be ionized. However electroosmotic water flow associated with iontophoresis

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can facilitate the transport of uncharged solutes (Gangarosa et al., 1980; Kim et al., 1993; Singh et al., 1995). Iontophoresis has been used to successfully deliver drugs locally (Russo et al., 1980; Tyle, 1986; Singh and Roberts, 1993). Examples abound on the use of transdermal iontophoresis in therapy (Grossi et al., 1986; Garagiola, et al., 1988; Meyer et al., 1992). The present focus of iontophoresis is on systemic delivery of compounds particularly proteins and peptide drugs (Singh and Maibach, 1994; Delgado-Charro and Guy, 1995).

An intrinsic part of the evaluations on any dosage form is the assessment of possible side/toxic effects. There is, however, limited information in the literature about the cutaneous effects of iontophoresis at the exposed sites (Ledger, 1992; Thysman et al., 1995; Van der Geest et al., 1996; Camel et al., 1996; Brand et al., 1997). The study reported here assesses non-invasively, skin irritation response and barrier function following iontophoretic treatments. The stratum corneum is the main structure responsible for the diffusional resistance of water. Measurements of transepidermal water loss (TEWL) across the stratum corneum is thus, thought to yield important information on the barrier function of the skin and its integrity at least to water (Van der Valk and Tupker, 1994). Skin irritation can produce an erythematous response. Changes in skin color were scored visually and measurements of subtle changes in dermal microcirculation at the treated sites were made using a Laser Doppler velocimeter. Studies have shown that rabbit skin is as reactive to applied chemical irritants as human skin after passive application (Phillips et al., 1972; Marzulli and Maibach, 1975; MacMillan et al., 1975; Franklin et al., 1991; Olson, 1991) and was therefore chosen as an animal model for these studies. At present, no accepted animal model exists for the assessment of iontophoretic protocols.

2. Materials and methods

2.1. Materials

2.1.1. Animals

Pathogen-free New Zealand rabbits purchased from Grimaud, Stockton, CA and housed under standard laboratory conditions (22 ± 1°C; relative humidity 40–55%) were fed with normal rabbit chow and watered ad lib. The animals when used ranged in age from 6 to 12 months and weighed between 7 and 10 lb. After each experiment, the animals were allowed a period of full recovery before being used for another study.

2.1.2. Other materials

Iontophoretic patches were obtained from Dermion Inc. (Salt Lake City, UT) and biocclusive dressings (Tegaderm™) from 3M Health Care, (St. Paul, MN). Analytical grade sodium chloride was purchased from Sigma Chemical Co. (St. Louis, MO). The power supply unit, hardware and software for iontophoresis were supplied by Novartis Pharmaceuticals Corporation (East Hanover, NJ).

2.2. Methods

The study was conducted according to a protocol approved by the Committee on Animal research at the University of California, San Francisco. In a pilot study, hair from the dorsal part of the animals was removed by depilation. As this resulted in severe irritation of the skin, the hair in all subsequent experiments were removed close to the skin surface using electric clippers. Areas of skin chosen for patch application were wiped with alcohol swabs. The animals were allowed to acclimatize for 30 min in restrainers before baseline measurements of transepidermal water loss (TEWL) and laser Doppler velocimetry (LDV). In addition, visual assessments of the extent of erythema and edema were made. Usually, the mean values for erythema and edema scores obtained after 24 and 72 h are combined to give primary irritation indexes but in this study, since edema was not always present, the responses were evaluated based on weighted scores and the mean values used individually to assess the extent of irritation when present.

The anode and cathode patches were made of silver and silver/silver chloride respectively. Each

patch was impregnated with 1.5 ml of normal saline, left to equilibrate and the pH measured. The patches were applied to the skin with the aid of templates made from the biocclusive dressings (Tegaderm™, 3M Co., St. Paul, MN.) exposing an area of either 1 or 4.5 cm^2. The anode and cathode patches were applied parallel to one another on the right dorsum separated by a distance of ≈ 3 cm as illustrated in Fig. 1. The leads from the patches were connected to the computer-controlled power source. Depending on the experimental design, the amount and duration of current to be applied were programmed into the computer. To ensure skin integrity, initial resistance of the skin was measured by passing a small current (20 $\mu A/cm^2$) for 18 s. A passive patch (no current) was applied to a site opposite to the anode/cathode to serve as a control.

The following parameters were varied for these experiments:

1. Current density: 0.1–4 mA/cm^2 (to identify the maximum tolerable current density in rabbit skin).
2. Duration: 1–4 h (to determine the influence of length of exposure to a specific current density on biological changes in the skin).
3. Area of patch application: 1 or 4.5 cm^2 (To determine if variations in area of exposure contribute to the observed effects).
4. Total charge-A constant amount of total charge (4 mA/cm^2 h) was delivered to the skin using two combinations of current density and application time through a fixed area of skin, 1 cm^2 (1 mA/cm^2 for 4 h and 4 mA/cm^2 for 1 h.)
5. Concentration of NaCl, 0.15 and 0.3 M (To assess the effects if any of ionic strength on the skin responses to electrical current).

2.2.1. Instrumental and clinical methods used in assessing skin irritation

Visual scoring: visual scoring of any erythema and edema was done using the following scale:

(A) Erythema and eschar formation: (0) no erythema; (1) very slight erythema (barely perceptible), either spotty or diffused; (2) moderate erythema; (3) intense erythema with papule formation; (4) severe erythema (beet redness) to slight eschar formation (injuries in depth).

(B) Edema formation: (0) no edema; (1) very slight edema (barely perceptible); (2) moderate edema (edges well defined by definite raising); (3) intense edema (area raised by about 1 mm); (4) severe edema (raised more than 1 mm and extending beyond area of exposure).

2.2.1.1. Transepidermal water loss (TEWL). TEWL values were recorded using an Evaporimeter EPI (Servomed, Vallingby, Sweden) in accor-

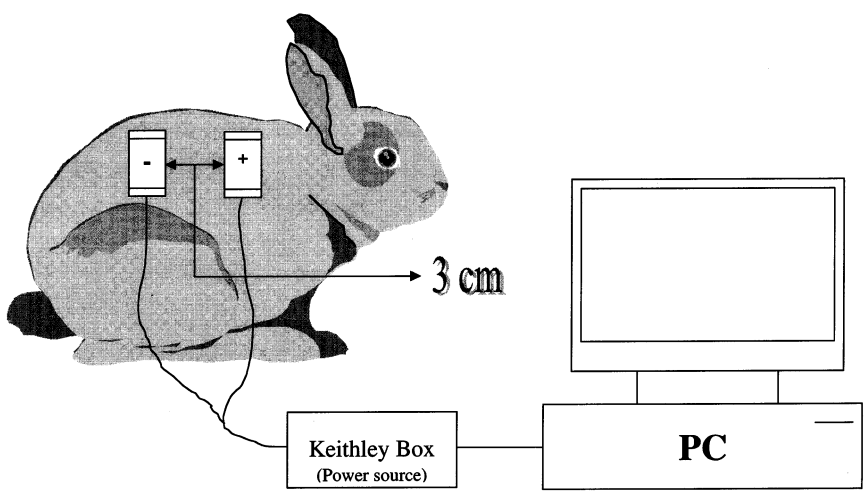

Fig. 1. A schematic representation of the transdermal iontophoresis set up used in the study.

dance with established guidelines (Serup, 1994). The method for evaporimetry uses the difference in the water vapor pressure gradient on the surface of the skin and has been described in detail (Nillson, 1977). The probe was held in position for 45–60 s before recording the TEWL values to attain a stable value at each site (Pinnagoda et al., 1989). The relative air humidity and room temperature were controlled and therefore fluctuations were negligible. The results obtained were the absolute values registered by the evaporimeter.

2.2.1.2. Laser Doppler velocimetry (LDV). Laser Doppler blood flow monitor (MBF3/D, Moor Instruments, Acaderm Inc., Menlo Park, CA) was used to measure skin blood flow according to established guidelines (Bircher et al., 1994). The mounting surface of the probe has a diameter of 1 mm. The LDV measures changes in microvascular perfusion, in terms of relative changes in blood volume and velocity. Measurements are based on the principle that when low-intensity laser light is focused on moving objects such as red blood cells, it undergoes a Doppler frequency shift, the amount of shift being dependent on the speed of the moving object. The back-scattered light is directed to photodetectors for conversion to electrical signal. The parameter most often used, the flux is related to the product of the speed and concentration of moving blood cells in the tissue sample volume and is expressed in perfusion units (PU). For the purpose of comparison, the results were expressed as the ratio of skin blood flow post iontophoresis to the pre-treatment value. Detailed description can be found in Bernardi and Berardesca (1995). In this study, perfusion measurements were made at three different spots on the patch application sites and the mean values taken.

2.2.1.3. Sequence of measurements. Before patch application, visual scoring was done first followed by measurement of TEWL and LDV. Following patch removal post-iontophoresis, the same sequence of measurements was repeated.

2.2.2. Statistical analyses

Statistical analyses of the data obtained pre- and post-treatment were done using Student's *t*-test on MS Excel. The data from the anode and cathode patch application sites were treated as ratios of post-iontophoresis to pre-iontophoresis values and compared to ratios of post-iontophoresis to pre-iontophoresis values from the control sites. The level of significance was taken as $P \leq 0.05$.

3. Results

In a preliminary study, chemical depilation of rabbit hair with two commercially available thioglycolate depilatory creams (Nair® and Surgicream®) was done with two objectives, to provide hair-free sites for patch application and to test for a positive irritant response. Both products caused severe irritation to rabbit skin with lesions appearing 24 h post-depilation and recovery of skin took about 2 weeks. Based on this, the idea of depilation was discarded and the animal hair was removed by clipping, close to the skin surface.

3.1. Effect of current density

For different protocols, currents of varying densities (0.1, 0.5, 1, 2 and 4 mA/cm^2) was applied to rabbit skin. At a current density range of 0.1–1.0 mA/cm^2 applied for 1 h to an area of 1 cm^2, no visual skin irritation was observed at either anode or cathode. In addition, the measured bioengineering parameters of TEWL and LDV showed only minor increases following current application. Compared to values measured at the control sites, none of the observed increases were significant and no further increases occurred in the days following treatment.

With the application of 2 mA/cm^2 current to an area of 1 cm^2 for 1h, slight erythema was observed on both the anode and cathode. No significant changes were observed in TEWL and LDV at either of the active electrode sites. The erythema on the anode was resolved within 24 h but persisted on the cathode and resolved 48 h post-iontophoresis. Following the delivery of 4 mA/

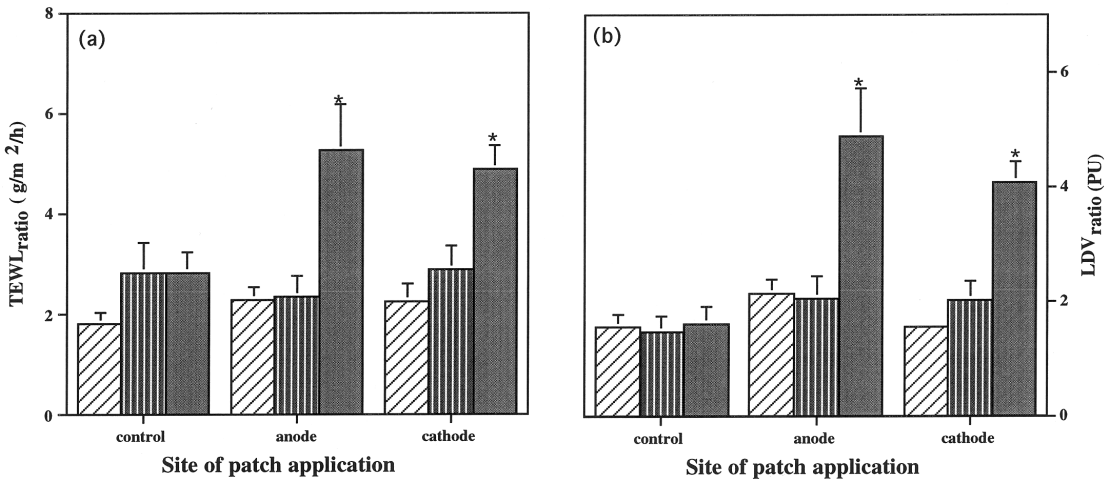

Fig. 2. The effect of current density on (a) TEWL and (b) LDV at patch application sites following iontophoresis. Area of application = 1 cm^2, duration 1 h, NaCl = 0.15M, pH = 7. Keys: (▨) 1 mA/cm^2; (▥) 2 mA/cm^2; and (▩) 4 mA/cm^2. Data represent mean ± s.d., $n = 4$, (* denotes statistical significance from control).

cm^2 current on an area of 1 cm^2 for 1 h, moderate erythema was observed at both the anode and cathode. Moderate edema was also observed at the anode. TEWL was significantly elevated at both anode and cathode ($P = 0.02$). The elevation in LDV was also statistically significant immediately following current delivery at both anode and cathode ($P = 0.01$).

Fig. 2 shows a comparison of the effect of current density on TEWL and LDV at the electrode sites. There was no significant difference between the responses to 1 and 2 mA/cm^2 at the active electrode sites and the control. There was however, a marked increase in TEWL in response to 4 mA/cm^2 current at both anode and cathode when compared to 1 and 2 mA/cm^2 currents. The LDV response following the passage of 4 mA/cm^2 was also greater than for 1 and 2 mA/cm^2 currents. The elevation in LDV at the anode after the application of 4 mA/cm^2 current was slightly higher than at the cathode. Brand et al. (1997) also observed greater skin blood flow at the anode than on cathode in human skin in vivo.

The profile of TEWL recovery post-iontophoresis following the application of 4 mA/cm^2 current density were comparable at the anode and cathode as shown in Fig. 3.

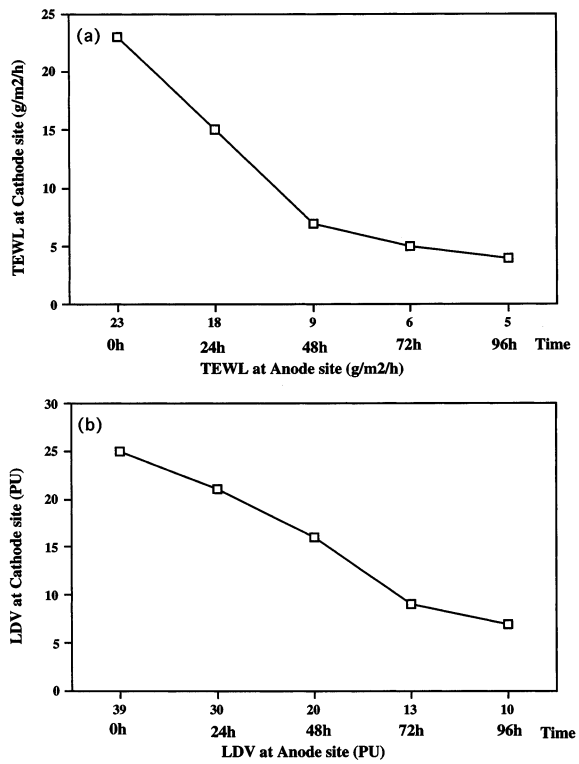

Fig. 3. A comparison of (a) TEWL and (b) LDV at the anode and cathode following iontophoresis. Current density = 4 mA/cm^2, area of application = 1 cm^2, duration = 1 h, NaCl = 0.15M, pH = 7. Data represent mean of four animals.

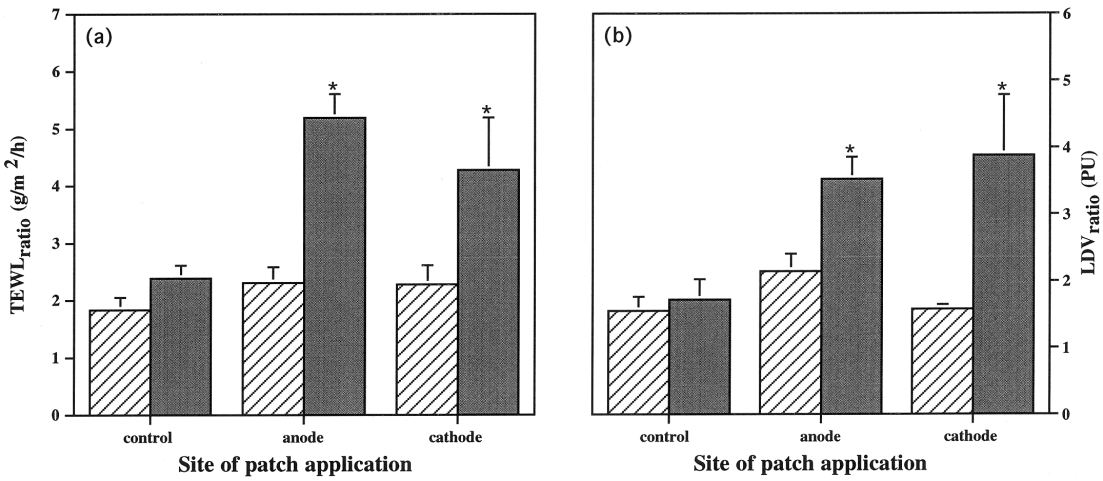

Fig. 4. Effect of duration of current application on (a) TEWL and (b) LDV following iontophoresis. Current density = 1 mA/cm^2, area of application = 1 cm^2, NaCl = 0.15M, pH = 7. Keys: (▨) 1 h; (▩) 4 h. Data represent mean ± s.d., $n = 4$ (* denotes statistical significance from control).

The response for LDV calculated as ratios of post/pre-treatment values was about 1.5 times higher at the anode than cathode. The erythema at the anode and cathode and the edema at the anode lessened with time and were resolved 48 h post-treatment. TEWL and LDV, however, remained significantly elevated until after 48 h and returned to near baseline values 3–5 days after treatment.

The $t_{50\%}$ (i.e. time taken for maximum recorded value to decrease by 50%) for TEWL following treatment with 4 mA/cm^2 was 36 h while for LDV, $t_{50\%}$ was 48 h. The resolution for TEWL and LDV were comparable at the anode and cathode.

From assessment of sensations, erythema and edema, human skin has been shown to tolerate up to 0.5 mA/cm^2 current for short periods (Banga and Chien, 1988; Burnette and Ongpipattanakul, 1988; Brand et al., 1997). It has also been shown that LDV but not TEWL increases in human skin in response to the application of 0.5 mA/cm^2 current for up to 30 min (Brand et al., 1997). From this study, rabbit skin showed more tolerance to electric current with no skin irritation being apparent with the application of 1 mA/cm^2 current for 1 h.

3.2. Effect of duration of current application

Fig. 4 shows a comparison of the TEWL and LDV responses at all sites immediately following the application of 1 mA/cm^2 current for 1 and 4 h periods. Small but insignificant elevations in the measured parameters were observed at the anode and cathode compared to the control site after 1 h application.

When 1 mA/cm^2 current was applied for 4 h, moderate erythema was observed at both anode and cathode. TEWL was significantly elevated immediately following current application ($P = 0.01$) at both the anode and cathode. Similarly, immediately following current delivery, LDV was significantly elevated at the anode and cathode ($P = 0.01$). The TEWL and LDV responses after 4 h current application were comparable at the anode and cathode. The $t_{50\%}$ for LDV at both anode and cathode was 24 h. The $t_{50\%}$ for TEWL was also 24 h at the anode and cathode.

3.3. Effect of area of application:

The effect of exposed area on skin response to iontophoresis, was examined in experiments which compared the delivery of 1 mA/cm^2 for 1 h

to skin areas of 1 and 4.5 cm^2 (i.e. total applied current was increased from 1 to 4.5 mA). The use of a relatively large surface area for current passage did not provoke a statistically significant difference in the responses as compared to smaller area.

3.4. Effect of applied total charge

A constant amount of total charge (4 mA/cm^2 h) was delivered to the skin using 1 mA/cm^2 current on an area of 1 cm^2 for 4 h or 4 mA/cm^2 current on an area of 1 cm^2 for 1 h. Moderate erythema was observed at both anode and cathode for both treatments while moderate edema was observed at the anode only when 4 mA/cm^2 was applied for 1 h. For both treatments, TEWL and LDV were significantly elevated at the active electrode sites immediately following current passage. A comparison of the TEWL and LDV responses to applied total charge under the two conditions immediately following current passage is represented in Fig. 5.

LDV response at the anode and cathode for the 1 mA/cm^2 treatment were comparable. LDV response was slightly higher at the anode than at the cathode for the 4 mA/cm^2 application. The LDV values at either anode or cathode were no longer significantly elevated 24 h post iontophoresis for the 1 mA/cm^2 treatment. For the 4 mA/cm^2 treatment, LDV remained significantly elevated for 48 h post-iontophoresis. TEWL response at the anode was slightly higher than on cathode following the delivery of 1 mA/cm^2 for 4 h. TEWL response at the anode and cathode following application of 4 mA/cm^2 for 1 h were comparable. TEWL responses for both treatments were comparable at the anode. Baseline TEWL values in rabbit skin is 4–5 g m^2/h. With occlusion, there is a 2–3 fold increase in TEWL. Recovery from occlusion occurs within 24 h post-treatment. Following the delivery of 1 mA/cm^2 current for 4 h, TEWL remained significantly elevated 24 h after treatment and was no longer significant 48 h post-iontophoresis. TEWL, following the application of 4 mA/cm^2 current for 1 h remained significantly elevated for 48 h post-iontophoresis and complete recovery took 4–5 days, which included the period of recovery from occlusion.

The $t_{50\%}$ for LDV and TEWL was 24h at both anode and cathode following the 1 mA/cm^2 treatment. For the 4 mA/cm^2 current, $t_{50\%}$ for TEWL was 36 h at both anode and cathode and for LDV, at both the anode and cathode, $t_{50\%}$ was 48 h and the recovery profile was the same for both parameters at both sites suggesting that the barrier alteration was to the same degree.

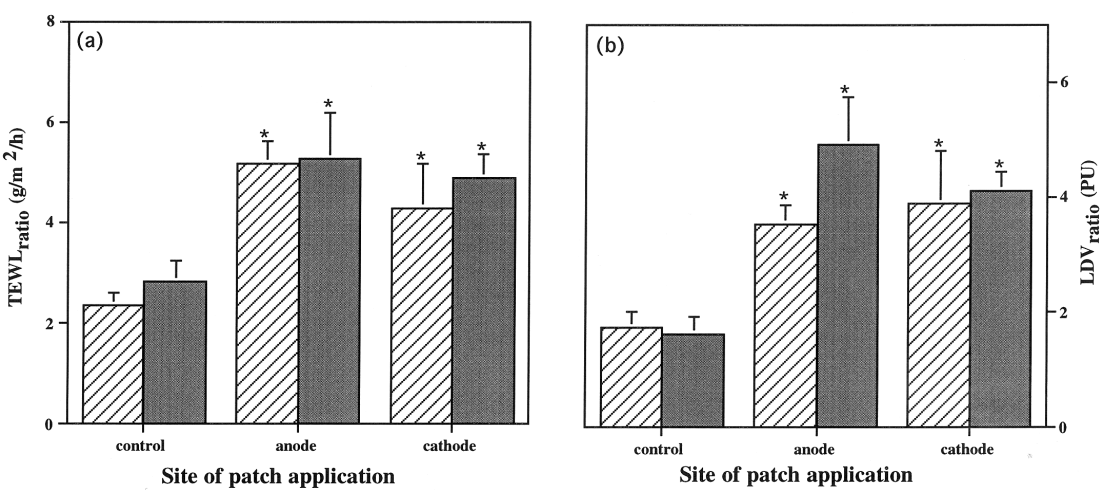

Fig. 5. A comparison of the effects of total applied charge on (a) TEWL and (b) LDV at patch application sites following iontophoresis. Area of appication = 1 cm^2, NaCl = 0.15M, pH = 7. Keys: (▨) 1 mA/cm^2, 4 h; and (▩) 4 mA/cm^2, 1 h. Data represent mean ± s.d., $n = 4$ (* denotes statistical significance from control).

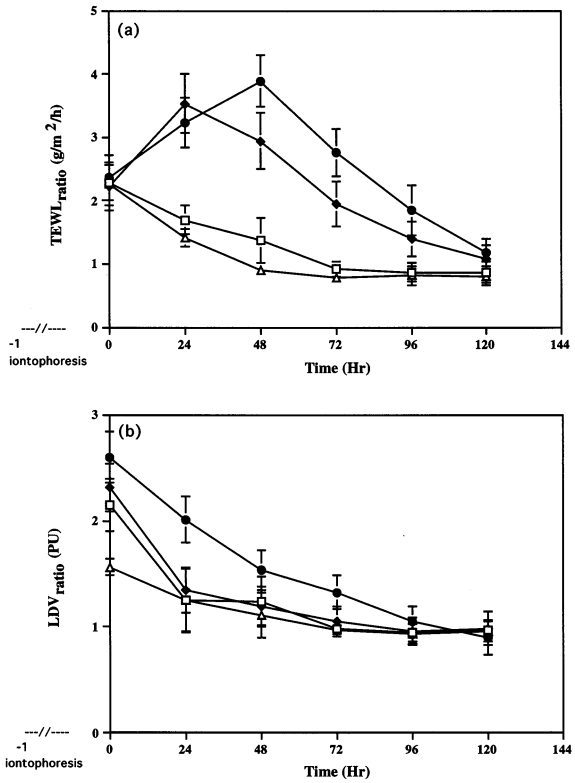

Fig. 6. The effect of salt concentration on (a) TEWL and (b) LDV at the anode and cathode following iontophoresis. Duration = 1 h, area of application = 4.5 cm^2. Keys: open symbols = 0.15 M NaCl; closed symbols = 0.3 M NaCl; (□) anode, (△) cathode, (●) anode, (◆) cathode. Data represent mean ± S.D., $n = 4$.

3.5. Effect of concentration

A current of 1 mA/cm^2 was applied to an area of 4.5 cm^2 for 1 h using solutions of NaCl having different concentrations, 0.15 and 0.3 M. Iontophoresis with normal saline did not produce any changes in the barrier integrity of the skin. Under the same conditions, a 0.3 M solution of NaCl caused moderate erythema on anode and cathode. LDV at anode and cathode were significantly elevated immediately following treatment ($P = 0.03$). The values at the cathode were considerably reduced 24 h post-treatment. The recovery at the anode was slower. TEWL at both anode and cathode were not significantly raised immediately following treatment. The TEWL responses at both sites were further elevated 24 h post-iontophoresis and significantly higher than control. The maximum response in TEWL to 0.3M NaCl was delayed as compared to other treatments. $TEWL_{max}$ at the cathode was observed 24 h post-iontophoresis, while at the anode, $TEWL_{max}$ was measured at 48 h. A comparison of the effect of salt concentration on TEWL and LDV at the active electrode sites is made on Fig. 6. Complete recovery following current application with 0.3M NaCl took 4–5 days, which included the period of recovery from occlusion.

3.5.1. Skin resistances

For all protocols, for skin integrity check, the initial resistance of the skin was measured by passing a small amount of current (20 μA/cm^2) for 18 s. The mean initial skin resistance was 60 ± 7 KΩ compared to human skin resistance which ranges from 100 to 10^3 KΩ. With increasing current delivery, the resistance of the skin fell until steady state values were attained. A profile of the typical change in skin resistance with time during constant current iontophoresis is shown in Fig. 7. For iontophoretic treatments with normal saline, exposing a skin area of 1 cm^2 for 1 h, a plot of current density against steady state resistance is shown in Fig. 8.

4. Discussion

Transepidermal water loss, which is considered a good indication of the functional status of the stratum corneum where the barrier of the skin is thought to reside, is actually a measure of the diffusion rate of water or water vapor across the skin. A damaged barrier will allow for greater loss of water vapor through the skin (Edwards and Marks, 1994). TEWL measurements have indeed been used to assess the barrier nature of the stratum corneum in disease states (Berardesca and Maibach, 1989) and in irritant-induced modifications of the stratum corneum (Tupker, 1994). It has been suggested however, that the increase in TEWL values observed following the iontophoresis process is mainly due to increased skin hydration from occlusion as well as local joule heating

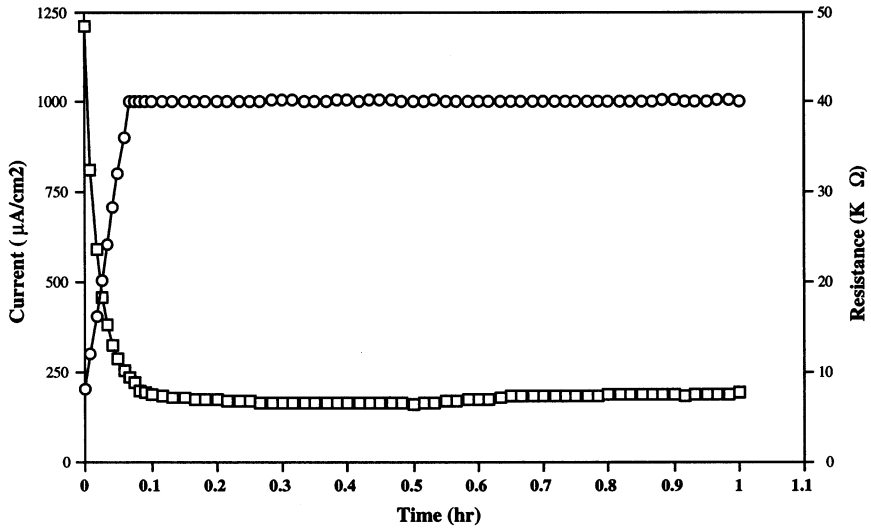

Fig. 7. A profile showing typical changes in skin resistance over time. Current density = 1 mA/cm^2, duration = 1 h, area of application = 1 cm^2, NaCl, 0.15M, pH = 7. Keys: (○) current; (□) resistance.

associated with the current (Thysman et al., 1995). While its effects cannot be ignored, our data analyses comparing the values from the iontophoresed sites to the passive control sites was designed to minimize the contribution of occlusion to the observed increases.

The minor changes observed in TEWL values following the application of 0.1–1 mA/cm^2 current over a 1 or 4.5 cm^2 area with normal saline were most likely due to an increase in the level of hydration of the stratum corneum. Increases of the same magnitude were recorded at the control electrode sites. The transient nature of the observed increases (recovery, usually within 24 h) does not suggest any irritation and concomitant barrier damage, which would have necessitated a longer recovery period. Similar observations have been made in vivo in humans (Thysman et al., 1995; Camel et al., 1996; Brand et al., 1997). In human studies, Van der Geest et al. (1996) and Brand et al. (1997) found a 2-fold increase in TEWL over baseline values at the passive electrode sites with the absolute TEWL value being about 13 and 15 g m^2/h respectively from both studies. In the studies reported here, following occlusion of rabbit skin for 1 h, there was a 2.5-fold increase in TEWL values with the absolute TEWL value being about 10 g m^2/h at the passive electrode site. Where obvious skin irritation was observed as in the passage of 4 mA/cm^2 for 1 h, the TEWL values were persistently elevated in the days following iontophoresis thus suggesting a breach in water barrier function.

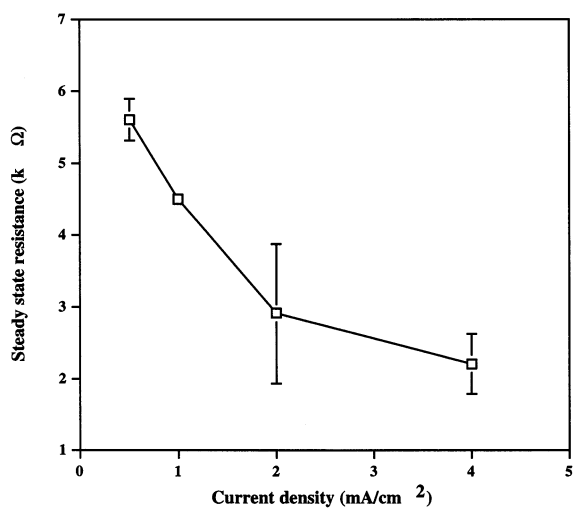

Fig. 8. Apparent steady-state skin resistance as a function of current density. Area of application = 1 cm^2, Duration = 1 h, NaCl = 0.15M, pH = 7. Data represent mean ± S.D., $n = 4$.

With the passage of 4 mA/cm^2 for 1 h through 1 cm^2 of skin, edema was observed at the anode upon cessation of current application. Edema is most likely due to accumulation of fluids underneath the skin. Edema may also have resulted from simple cell damage and leakage. Low to moderate voltage iontophoresis has been shown to induce reversible pores with sizes of the same order of magnitude as pre-existing pores in human epidermal membrane (Inada et al., 1994; Li et al., 1998). The application of higher current densities on rabbit skin may have caused transient pores to be created in the skin with a resultant increase in the transport of water into the skin under the anode. The skin is a permselective membrane and carries a net negative charge at physiological pH (Burnette and Ongpipattanakul, 1987). With the establishment of an electrical potential gradient across the skin during iontophoresis, Na^+ will be driven by electrostatic repulsion at anode. Similarly, anions such as Cl^- are repulsed but to a lesser degree at the cathode. In addition, electroosmotic flow in the same direction as flow of the counterions may occur preferentially at the anode (Pikal, 1992). The overall effect may be an increase in Na^+ transport along with water resulting in edema at the anode.

The pain threshold of humans to electric currents as a function of exposed area has been measured and it was found that the maximum tolerable current increased with increased area in a nonlinear fashion (Molitor and Fernandez, 1939). Although this study did not use larger surface areas than 4.5 cm^2, it was seen that increasing the surface area of the skin exposed to the same current density and in effect increasing the total amount of current delivered, did not cause increases in the measured skin responses as well as changes in visual observations.

Even though the total charge delivered was the same, the recovery of the skin was faster after the 1 mA/cm^2 current application for 4 h, taking 48 h than with 4 mA/cm^2 current delivery for 1 h which took 72 h. The implication of this observation is that in considering the appropriate protocol to be adopted for iontophoresis, it is more advantageous for less current to be applied over a longer time period as this reduces the time required for skin recovery.

The physical appearance of the skin as well as the values of the measured parameters used in this study suggest no skin irritation or barrier alteration arising from the use of small currents with normal saline. In contrast, however, a solution of NaCl having a higher concentration (0.3 M) did produce irritation at 1 mA/cm^2 current.

The skin constitutes a large diffusional resistance to the transport of charged molecules under the influence of an applied electrical field. In this study, the skin resistance declined during prolonged delivery of direct current with the decline being most rapid at the start of current application. Thereafter, the drop in resistance was less dramatic and subsequently, a steady state was reached. Similar profiles in resistance with electric current application have been reported by others (Sims et al., 1992; Craane-van Hinsberg et al., 1994, 1995). Changes in the molecular arrangements of the intercellular lipids in the stratum corneum where the barrier of the skin is thought to reside may be responsible for the initial rapid drop in resistance. The subsequent less dramatic decrease in skin resistance could be due to an accumulation of excess ions in the skin.

The elevated LDV values following the iontophoretic treatments suggest an increase in the activities at the dermal vascular bed as a result of the applied electric current. Moreover, the absence of erythema on any of the passive electrode sites suggests that occlusion does not contribute to the increased blood flow. Immediately following treatment with hypertonic NaCl solution or application of 4 mA/cm^2 current, there was good correlation between the visual score and LDV measurements illustrating the complementarity of the two methods in detecting skin irritation. However, this correlation, did not extend to later time points, as the visually observed erythema at the active electrode sites tended to be less obvious within 24–48 h of treatment, while the measured LDV values at the sites remained significantly elevated for several days following the iontophoretic treatments suggesting that different mechanisms may be in operation at different time points.

The erythema observed immediately after iontophoresis may be due to a direct effect of electric

current on cutaneous blood vessels and/or current-induced release of histamine, prostaglandins or other neurotransmitters leading to local vasodilatation in the affected area. It has also been suggested that electric current can stimulate specific classes of noiceptors, the C-fibers causing them to release the potent vasodilators, substance P and calcitonin gene-related peptide (CGRP) (Brain and Edwardson, 1989; Dalsgaard et al., 1989). The persistence of high LDV values at the iontophoresed sites several days after certain iontophoretic treatments cannot be explained by simple joule heating alone or release of histamine or other vasodilators which would have caused a transient increase in skin blood flow. There thus appears to be cellular changes resulting from such treatments (the passage of current of high density and use of hypertonic solutions in the patches). It is, however, not possible from the present study to offer an explanation as to the nature of the molecular events occurring within the skin under these conditions that may be responsible for the observed increase in activities at the dermal vascular bed. Overall, from this study neither of the active electrode sites can be deemed the most responsive to iontophoresis as the responses varied with different treatments.

From the results presented here, it is seen that the effects of small amounts of electric current (0.1–1 mA/cm^2) used with normal saline on rabbit skin as assessed by transepidermal water loss and responses from the vasculature were minimal suggesting that the barrier of the skin is intact under such conditions. Higher current densities as well as sodium chloride solutions of higher concentration produce significant but transient irritation in rabbit skin. Rabbit skin was able to discriminate between irritating and non-irritating conditions for iontophoretic treatments but needs more work for development as a suitable model for man.

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