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Electrically enhanced transdermal delivery of a macromolecule

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Abstract

The purpose of this study was to establish the delivery parameters for the enhanced transdermal delivery of dextran sulfate (MW 5000 Da). Full-thickness pig skin or epidermis separated from human cadaver skin was used. Silver–silver chloride electrodes were used to deliver the current (0.5 mA cm^{-2}). For electroporation experiments, one or more pulses were given using an exponential decay pulse generator. The correct polarity for iontophoresis and pulsing was first established as cathode in the donor. The amount of drug delivered increased with increasing donor concentration up to a point, but not any further. The amount delivered also increased with pulse voltage, the delivery being twice as much as with iontophoresis alone (144.5±10.35 μg cm^{-2}), when 6 pulses of 500 V were applied at time zero before iontophoresis (276±45.2 μg cm^{-2}). It was observed that the amount delivered was a function of increasing pulse length when the apparent charge delivered was kept constant. Transport through pig skin (107.4±24.4 μg cm^{-2}) was found to be comparable with that through human epidermis (84.9±18.4 μg cm^{-2}). In conclusion, we have demonstrated the transdermal delivery of a 5000 Da molecular weight dextran sulfate using iontophoresis. It was also seen that iontophoretic delivery could be enhanced by simultaneous electroporation.

Introduction

Biologically active macromolecules, like peptides or proteins, generally have no, or very low, bioavailability, making oral administration difficult. They also have a short half-life, rendering parenteral administration impractical outside the hospital setting. Alternative routes of administration have been explored and one of the most promising is the transdermal route. By itself or in combination with chemical enhancers, transdermal delivery can achieve therapeutic levels only for potent lipophilic drugs with a low molecular weight. This is because only limited quantities of small molecules with sufficient solubility in skin lipids can pass through the skin, exhibiting lag times of hours to days and steady-state rates which are often sub-therapeutic. Iontophoresis offers a unique opportunity to expand the scope of transdermal delivery to include the systemic delivery of hydrophilic and charged macromolecules such as proteins, peptides, oligonucleotides and sulfated polysaccharides. Iontophoresis involves the application of a small amount of physiologically acceptable direct current to drive ionic drugs into the body (Green et al 1993; Sage 1993). Transdermal iontophoretic systems are currently in clinical trials and are likely to be available in the near future as disposable, battery-operated wristwatch-type devices, controlled by microchips to deliver drugs at the desired rate (Banga 1998).

Electroporation is another technique which can be used, by itself or in combination with iontophoresis, to enhance transdermal drug delivery. It involves the use of a high voltage, electric pulse for a very short period of time (μs to ms duration). This causes the rearrangement of lipid bilayers in the stratum corneum (the outer-most layer of the skin), which leads to increased permeability (Weaver 1995; Weaver & Chimadzhev 1996; Banga & Prausnitz 1998). This technique is routinely used on the unilamellar phospholipid bilayers of cell membranes to transfect cells and to introduce DNA inside them. However, it has been demonstrated that electroporation of skin is feasible, even

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though the stratum corneum contains multilamellar, intercellular lipid bilayers with no phospholipids (Prausnitz et al 1993; Prausnitz 1999).

Comparisons and contrasts between iontophoresis and electroporation have been reviewed (Banga et al 1999). The use of electroporation in conjunction with iontophoresis can expand the scope of transdermal delivery to larger molecules and has been reported in the literature for gonadorelin (LHRH) (Bommannan et al 1994), colchicine encapsulated in liposomes (Badkar et al 1999) and calcium-regulating hormones (Chang et al 2000). The safety of iontophoresis and electroporation has been reviewed and these techniques are considered to be safe at the settings typically used for transdermal delivery (Jadoul et al 1999; Pliquett & Gusbeth 2000; Curdy et al 2001).

In this study, the macromolecule investigated was dextran sulfate (MW 5000 Da), a sulfated polymer of anhydroglucose possessing a high negative charge. This makes it very similar in size, charge and other physical properties such as its hydrophilic nature to most antisense oligonucleotides. Antisense oligonucleotides have the ability to selectively block disease-causing genes, thereby inhibiting the production of disease-associated proteins, and are in clinical trials in man for a variety of disorders (Nolen et al 1994). Several complications are being faced in the delivery of these oligonucleotides, like degradation in the gastrointestinal tract, need for continuous administration, extensive first-pass metabolism and, in some cases, a need for pulsatile delivery. The transdermal route offers a non-invasive method for delivering these drugs and bypasses most of the aforementioned hindrances. The objective of this study was to use dextran sulfate as a model macromolecule and check the feasibility of its electrically assisted transdermal delivery using iontophoresis and electroporation and a combination of both.

Materials and Methods

Materials

Dextran sulfate (MW 5000 Da) and ^{14}C-labelled dextran sulfate of the same molecular weight were obtained from Sigma Chemical Company (St Louis, MO). Scintillation fluid (ULTIMA-GOLD) and Solvable tissue and gel solubilizer were obtained from Packard (Meriden, CT). Human cadaver skin was obtained from a skin bank. The skin was frozen within 12 h of death and supplied as full-thickness skin. Once received, the skin was stored at -80°C and thawed just before use. The study was approved by the Institutional Review Board. Pig skin was obtained from Auburn University, College of Veterinary Medicine (Auburn, AL). The pigs were neonatal (15 days old) Genus species, Sus scrofa. The abdominal skin was removed and frozen at -80°C and thawed just before use.

Preparation of skin

Human epidermis was prepared by heating full-thickness cadaver skin in water at 60°C for 45 s, and rubbing gently with two spatulas (Steinstrasser & Merkle 1995). The epidermal membrane was then teased off the underlying dermis by forceps and was spread over a piece of parafilm to facilitate cutting into appropriately sized pieces which were mounted between the donor and receptor halves of the diffusion cells. The pig skin was thawed by immersion in a beaker of distilled water at room temperature. The underlying subcutaneous fat was gently scraped off. The resulting skin was about 1–1.5 mm thick. The skin was cut into appropriately sized pieces and mounted between the diffusion cells.

Transdermal iontophoretic studies

Prepared skin samples were mounted on the side-by-side Valia-Chien (VC) cells, modified to have two ports on each half, and having a diffusional area of 0.64 square cm. One port served as the sampling port while the other accommodated the electrode. Silver–silver chloride electrodes were used as they are reversible and do not cause electrolysis of water which may result in pH shifts. A silver wire was used as the anode and a silver–silver chloride matrix was used as the cathode. Unless otherwise specified, the cathode was placed in the donor solution, which contained 4 mL of the specified concentration of dextran sulfate (MW 5000), spiked with 0.5 l Ci mL^{-1} of ^{14}C-labelled dextran sulfate, in 25 mM HEPES buffer. The HEPES buffer contained HEPES salt, HEPES acid, sodium azide and sodium chloride in distilled water. The pH of 7.4 ± 0.05 was adjusted with tetrabutylammonium hydroxide. The receptor phase was filled with plain HEPES buffer (pH 7.4 ± 0.05). A current of 0.32 mA (0.5 mA cm^{-2}) was applied for 6 h unless otherwise specified. Sampling was continued for 24 h. Samples of 1.0 mL were withdrawn at specified intervals and replaced with 1.0 mL of fresh HEPES buffer.

Transdermal electroporation studies

The prepared skin samples were mounted on the VC cells as described earlier. Platinum wires, which were used as electrodes for pulsing, were placed in the inner ports of the cells. The donor chamber was filled with 4 mL of the specified concentration of dextran sulfate (MW 5000), spiked with 0.5 l Ci mL^{-1} of ^{14}C-labelled dextran sulfate in 25 mM HEPES buffer at pH 7.4 ± 0.05. The receptor was filled with plain HEPES buffer at the same pH. High-voltage (100 V) pulses were applied to the skin. The voltage of pulsing and the pulse length could be varied. The pulse rate (number of pulses per minute) was controlled manually using a stop-watch. For multiple pulsing at time zero, the pulses were given 6 s apart. The electrode polarity during pulsing was as specified. Samples of 1.0 mL were withdrawn at specified intervals and replaced with 1.0 mL of fresh HEPES buffer.

Studies using iontophoresis and electroporation

For these studies, the skin was mounted as described earlier. The outer ports of the VC cells were used for iontophoresis and the inner ports for electroporation. Silver–silver

chloride electrodes were used for iontophoresis whereas platinum electrodes were used for pulsing (electroporation). The iontophoresis current was always stopped and the connection disabled while pulsing to prevent any potential damage to the iontophoresis equipment from the high voltage generated by the electroporation equipment. Iontophoresis current was immediately applied or resumed after pulsing. Samples were collected at specified intervals and replaced with 1 mL of fresh buffer.

Charge measurements

Comparing the efficiency of iontophoresis and electroporation was done by comparing the amount of drug transported with respect to the total charge (Q) delivered across the skin. In the case of iontophoresis the total charge is defined as the applied current (I_a) in amperes multiplied by the total on-time (t) in seconds:

$$Q = I_a \times At \quad (1)$$

In the case of electroporation, the total charge delivered is given as:

$$Q = I_p \times s \times n \quad (2)$$

where I_p is the current passing through the skin during the pulse, s is the pulse length (s) and n is the number of pulses (Prausnitz et al 1996a). The current during the pulse was recorded using an oscilloscope, and s was calculated by capturing the pulse and later analyzing it using software (WaveStar).

Quantitative analysis of drug in the skin and receptor

At the end of most experiments, the skin was removed, washed with buffer, and digested with 5 mL Solvable per gram of skin. This mixture was incubated in a metabolic shaking incubator at 70°C until the skin had completely dissolved. Typical times for the skin to dissolve were 1–2 days for epidermis and 2–4 days for full-thickness skin. The residual radioactivity in the skin was assayed. To 1 mL of sample (either receptor or dissolved skin samples), 2 mL of Ultima-Gold scintillation fluid was added. This was then allowed to sit for 2–3 h. Radioactivity in these samples was measured using liquid scintillation counting. All values were expressed as mean ± s.d. When comparisons between experiments were conducted, significant differences were assessed by Student's *t*-test (unpaired) and one-way analysis of variance.

Results and Discussion

Dextran sulfate (MW 5000 Da) was used as a model drug to establish the ideal conditions and parameters for electrically assisted transdermal delivery. We first used full-thickness pig skin to study various parameters and then compared the delivery with that across human epidermis. For iontophoresis, a current density of 0.5 mA cm^{-2} was used as it is well tolerated by patients (Panus et al 1996). Hence in all iontophoresis experiments, the current density was maintained at 0.5 mA cm^{-2}. We studied the effect of electrode polarity on the transdermal delivery of dextran sulfate (donor concentration 1 mg mL^{-1}) during iontophoresis (6 h). Since there are two mechanisms involved during iontophoresis, electrorepulsion and electroosmosis, it was important to find out which one was more significantly responsible for enhancement of transport in this particular case. Figure 1 shows the average cumulative amount present in the receptor during cathodic, anodic and passive delivery. The amount delivered under cathode at the end of 8 h was much higher (98 ± 26.4 l g cm^{-2}) than that delivered under anode (4 ± 1.14 l g cm^{-2}) or passively (0.3 ± 0.38 l g cm^{-2}). Thus, it was seen that electrorepulsion (which is the main contributing mechanism in the case of cathodic delivery) was the primary mechanism as opposed to electroosmosis (which is the main mechanism involved in anodic iontophoresis of a negatively charged drug) for dextran sulfate. We used cathodic iontophoresis for all further experiments.

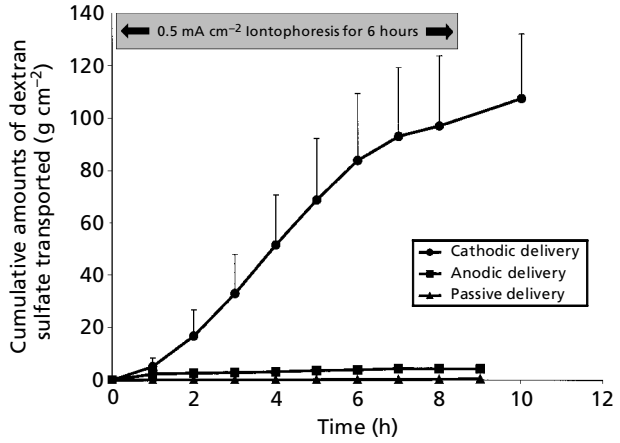

Figure 1 Effect of electrode polarity on iontophoretic delivery of dextran sulfate across full-thickness pig skin (bars represent s.d.).

Next we looked at the effect of donor concentration on the iontophoretic transport of dextran sulfate. Figure 2 shows the average cumulative amount present in the receptor and the amount accumulated in the skin versus time as a function of increasing donor concentration. The cumulative amount transported across the skin increased with an increase in donor concentration up to a certain point, beyond which it plateaued. This is in agreement with the literature reports (Miller et al 1990; Lu et al 1993). The reason for this non-linear behaviour of flux with increasing donor concentration is not entirely clear. However, the amount present in the skin also increases with increase in donor concentration. Hence it is plausible that an interaction might be taking place between the skin and dextran sulfate (i.e., it might be binding to the skin at certain regions and blocking skin appendages through which iontophoretic transport takes place). Similar drug–skin interactions have been reported for heparin (Prausnitz et al 1995). The initial increase in amount of dextran sulfate

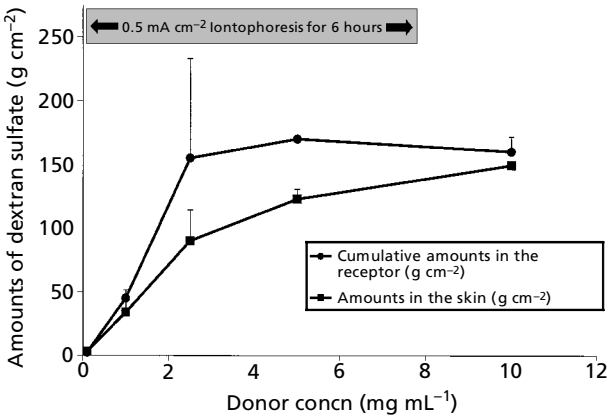

Figure 2 Effect of donor concentration on the transdermal transport of dextran sulfate across full-thickness pig skin during iontophoresis (bars represent s.d.).

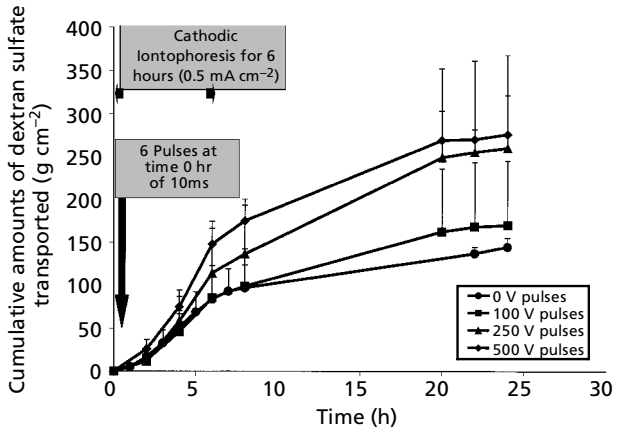

Figure 4 Effect of electroporation on iontophoretic delivery of dextran sulfate through pig skin (bars represent s.d.).

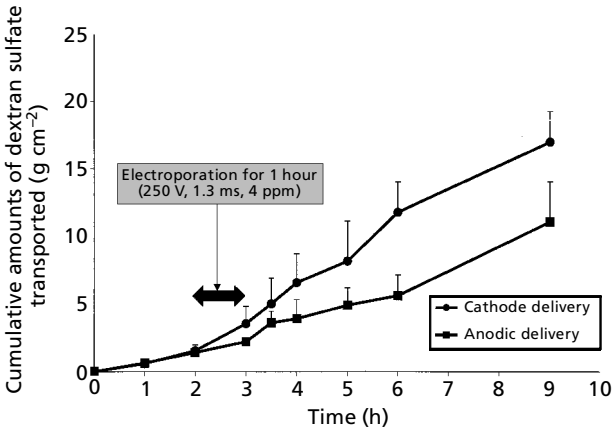

Figure 3 Effect of electrode polarity during electroporation on transdermal delivery of dextran sulfate across full-thickness pig skin (bars represent s.d.).

being transported with an increase in donor concentration can be explained according to the Nerst–Planck equation, which states that the flux of drug ions across a membrane is directly proportional to the concentration of the drug ions in the solution.

The effect of electrode polarity on transdermal transport during electroporation is seen in Figure 3. The experiment was run passively for 2 h before pulsing. Pulsing was carried out at 250 V, with each pulse lasting 1.3 ms, at a rate of 4 pulses per min, for 1 h. After this the cells were allowed to run passively for 6 h for a total of 9 h. The cumulative amount transported across was greater when the cathode was in the donor (17 ± 2.2 l g cm^{-2}) as compared with when the anode was in the donor (11 ± 3 l g cm^{-2}). This shows that pulsing with forward polarity gives slightly better results. Though structural changes in the skin take place during pulsing, it is possible that the electrostatic repulsive forces, which provide an electrophoretic driving force for the negatively charged dextran sulfate during the pulsing, also contribute a little towards the flux enhancement during electroporation. Thus, for all further experiments involving pulsing of the skin, the electrodes were placed according to forward direction polarity (i.e., cathode in the donor).

To investigate whether the pulse voltage affects the transdermal permeation of dextran sulfate, 6 pulses of 10 ms each were applied at time zero. A pulse voltage of 100, 250 or 500 V was applied, followed by iontophoresis at 0.5 mA cm^{-2} for 6 h. Figure 4 shows the average cumulative amount transported through the skin as a function of pulse voltage. As the applied voltage increased the amount transported also increased. There was no statistically significant difference between pulsing at 250 V and pulsing at 500 V, and pulsing at 100 V and only iontophoresis, but there was a significant difference between pulsing at 500 V and only iontophoresis, and 250 V and only iontophoresis ($P < 0.01$). The effect of pulsing voltage on the reversibility of the skin was noted by observing the flux of dextran sulfate during (first 6 h) and after iontophoresis (after hour 6). During iontophoresis, it was observed that the flux increased as a function of increasing pulsing voltage. When the iontophoretic current was stopped, after 6 h, the cells which had not been subjected to pulsing showed an immediate decline in flux from 18.58 l g h^{-1} to 4 l g h^{-1} ($P < 0.01$), whereas those cells which had been subjected to pulsing showed elevated fluxes even after iontophoresis had been stopped. The post-iontophoretic flux was highest when the pulsing voltage was 500 V and lowest when the pulsing voltage was 100 V. Thus, it may be concluded that the structural changes formed in the skin as a result of electroporation are voltage dependent. The higher the pulsing voltage, the more intense are the structural changes and, hence, the more time required for the skin to recover.

Prausnitz et al (1996a) showed that, over a range of conditions, transport of a model compound, calcein, was approximately proportional to total pulse-on time (i.e., the product of pulse length multiplied by the pulse rate). We also have studied the effect of pulse length and the frequency of pulsing, keeping the total apparent charge delivered and

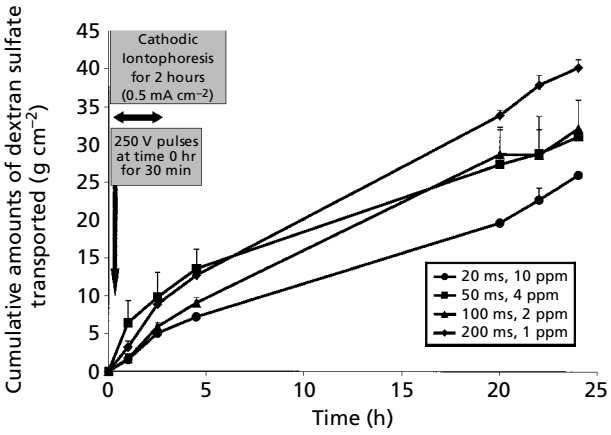

Figure 5 Effect of pulse length and pulse frequency on iontophoretic delivery of dextran sulfate through pig skin (bars represent s.d.).

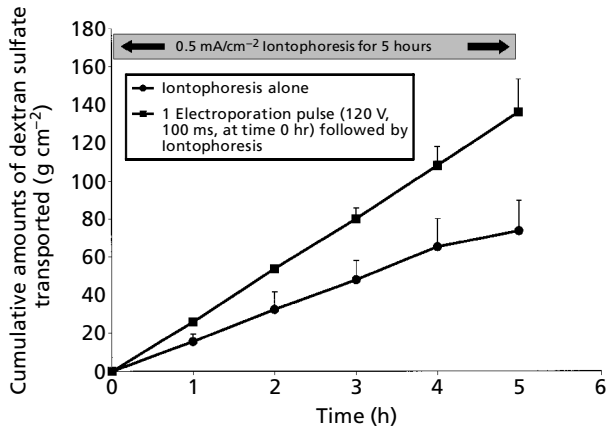

Figure 7 Effect of electroporation on the iontophoretic delivery of dextran sulfate through human epidermis (bars represent s.d.).

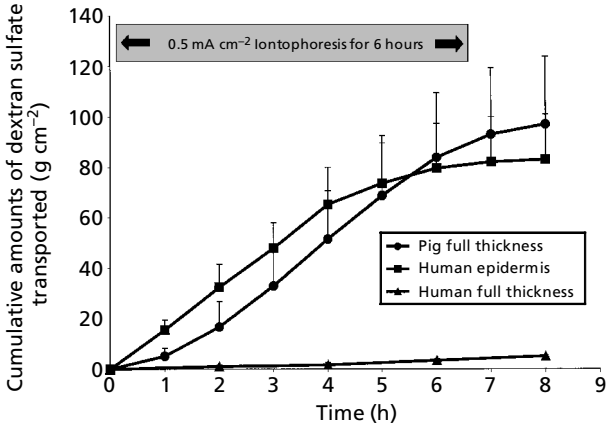

Figure 6 Effect of iontophoresis on transdermal delivery of dextran sulfate through pig full-thickness skin, human epidermis and human full-thickness skin (bars represent s.d.).

the total pulse-on time constant in all cases. We used four pulse lengths (20, 50, 100 and 200 ms) with corresponding pulsing frequency (10, 4, 2 and 1 pulses per min, respectively). Thus, the product of pulse length and pulse rate was constant (200 ms × pulses per min). The pulsing voltage used was 250 V. Pulsing was executed at time zero and followed by iontophoresis (0.5 mA cm^{-2}) for 2 h. Figure 5 shows that the longest pulse length (200 ms) resulted in the highest cumulative amount of dextran sulfate transported at the end of 24 h. The shortest pulse length (20 ms) showed the least amount transported at the end of 24 h. The difference in amount transported over 24 h between 50 ms, 4 pulses per min and 100 ms, 2 pulses per min was statistically insignificant. This was higher than that for 20 ms, 10 pulses per min and lower than that for 200 ms, 1 pulse per min. It has been speculated that pathways created in the skin during electroporation grow in size during a pulse (i.e., longer pulses are associated with larger pores) (Prausnitz et al 1996b). It is also known that as the frequency of pulsing increases, the skin has less time to recover completely before it is subjected to another pulse. Hence, the structural changes which are caused in the skin are more intense. In our case, the pulse length seems to play a major role as compared with the pulse frequency.

All the studies performed thus far had used full-thickness pig skin. To see how this permeation would compare with that of full-thickness cadaver skin and epidermis from man, we performed an experiment involving iontophoresis of all three types of skin (Figure 6). Full-thickness human skin showed very low permeability, whereas any differences in the profiles shown by human epidermis and full-thickness pig skin were statistically insignificant ($P > 0.01$). Human skin and pig skin has similar hair-follicle density, around 11 hair follicles/square cm of skin (Banga 1998). Since shunt pathway is important in electrotransport, this, along with other structural similarities, may explain the close correlation seen for human epidermis and pig skin. The permeability for full-thickness human skin is lower as it is much thicker. In physiological situations, human epidermis would be a better model since the blood circulation lies under the epidermis and the drug does not have to transport across full thickness skin.

Figure 7 shows the effect of pulsing on the iontophoretic delivery of dextran sulfate using human epidermis. A single pulse of 120 V for 100 ms was given at time zero, followed by iontophoresis for 5 h. The control in this case was only iontophoresis (0.32 mA or 0.5 mA cm^{-2}) for 5 h. The total charge delivered across the skin when the single pulse of 120 V was given for 100 ms was calculated using Equation 2. The current passing through skin was measured to be 120 mA and measured pulse length was 80 ms. The corresponding charge was calculated to be 9.6×10^{-3} Coulombs. The charge delivered during iontophoresis was calculated by Equation 1 to be 5.76 Coulombs. Hence the total charge delivered during the combination of electroporation and iontophoresis was 5.7696 Coulombs and that during iontophoresis alone was 5.76 Coulombs. Thus by adding only a small fraction of charge (during pulsing), the iontophoretic transport was nearly doubled. This sug-

gests that enhancement by electroporation is not due to charge alone and gives support to the idea that electroporation causes structural changes in the skin, which in turn leads to increased transdermal transport.

Conclusions

The feasibility of transdermal delivery of dextran sulfate (MW 5000 Da) has been shown. There was little or no passive flux across skin, and iontophoretic delivery was most efficient when the cathode was in the donor. This suggests that electrostatic repulsive forces play a significant role in the mechanism of delivery. It was found that delivery under cathode was higher than that under anode. Increasing pulse voltage increased the delivery across skin. It was seen that the amount delivered increased non-linearly with increasing pulse length when the total pulse-on time was kept constant. Pig skin was comparable with human epidermis in its permeability. A single pulse before iontophoresis was found to increase the iontophoretic transport of dextran sulfate to nearly twice that of iontophoresis alone. This result supports the hypothesis that it is not only the charge but also, more importantly, the structural rearrangement of the lipid bilayers which lead to enhanced permeability and thus more transport.

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