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Review

Electrically-Assisted Transdermal Drug Delivery

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Electrically-assisted transdermal delivery (EATDD) is the facilitated transport of compounds across the skin using an electromotive force. It has been extensively explored as a potential means for delivering peptides and other hydrophilic, acid-labile or orally unstable products of biotechnology. The predominant mechanism for delivery is iontophoresis, although electroosmosis and electroporation have also been investigated. The focus of this review is to put these different mechanisms in perspective and relate them to the drug and skin model system being investigated.

KEY WORDS: iontophoresis; electrically-assisted delivery; transdermal; electroosmosis; electroporation.

INTRODUCTION

Electrically-assisted transdermal delivery (EATDD) is the facilitated transport of compounds across the skin using an electromotive force. Depending on the nature of the applied electrical field, the molecule to be delivered and the barrier to be crossed, this mode of active delivery includes iontophoresis, electroosmosis, iontohydrokinesis, electroporation and electroincorporation. EATDD presents a novel technique for the transdermal delivery of compounds both locally into the skin as well as into the systemic circulation.

The skin's outermost layer, the stratum corneum, is generally thought to contribute most to its barrier function. Passive penetration of this layer is especially difficult for compounds which are charged or are not lipophilic, properties which are characteristic of many of the peptide and oligonucleotide drugs produced by the biotechnology industry. EATDD enhances the transdermal delivery of compounds which, because they are large, hydrophilic or charged, are unable to penetrate the skin under passive conditions. With iontophoretic delivery, the charged species are contained in an electrolyte solution and the generated electric field forces charged molecules to move down their electromotive gradient. With electroosmosis or iontohydrokinesis, the electric field creates a convective flow of water which allows hydrophilic compounds to be transported. Electroporation and electroincorporation, which transiently apply high voltage gradients across the skin, momentarily decrease the skin's electrical resistance thereby increasing its permeability to normally restricted compounds. These latter two approaches do not provide a driving force for delivery but rather reduce the barrier to delivery (increase permeability). They require a chemical concentration gradient or a simultaneously applied lower electrical current to provide the driving force to tranverse the stratum corneum barrier. Presently, most EATTD systems under development utilize iontophoretic delivery and thus this mode will be the focus of this paper.

The best approach to understanding iontophoresis is to consider it in its simplist form, the iontophoretic cell depicted in Figure 1. The cell consists of donor and receptor solutions separated by a limiting membrane across which a voltage gradient is generated by connecting an anode and cathode to a voltage source. For delivery of positive ions (cations), the anode (positive electrode) is placed in the donor compartment and for delivery of negative ions (anions), the cathode (negative electrode) is placed in the donor side. The voltage source most often supplies a constant direct electronic current which is converted by oxidation-reduction reactions at the electrodes to an ionic current. As ions carry this current through the barrier membrane, the circuit is completed. In most situations, the electrolyte solution contains several ionic species which may carry current. As far as current carrying ability is concerned, the movement of a positive ion of $+1$ charge moving from donor to receptor is equivalent to a -1 ion moving from the receptor to donor compartment. When water dipoles carry the applied charge, the resulting convective flow is the driving force of electroosmosis.

The idea of applying an electric current to enhance the penetration of a charged compound into a tissue probably originated by Veratti in 1747. The first well-documented experiments using iontophoresis were performed at the beginning of the 20th Century by LeDuc (1). A positively charged ion, strychnine sulfate, caused tetanic convulsions in a rabbit when placed in the positive electrode but had no effect from the negative electrode. Conversely, potassium cyanide, a negative ion, caused cyanide poisoning when placed in the negative electrode, but had no effect from the positive electrode. Although these experiments would no doubt have difficulty passing a modern institutional animal care committee, this remains a dramatic

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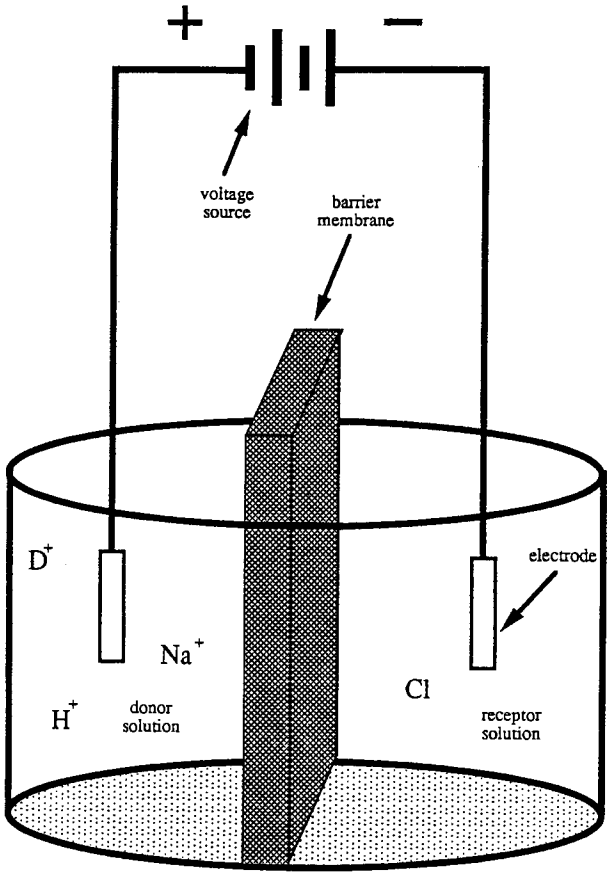

Fig. 1. Schematic representation of an iontophoretic cell.

illlustration of the selectivity and control inherent to iontophoretic drug delivery.

THE BARRIER

An iontophoretic cell necessitates a barrier which separates donor and receptor compartments. In *in vitro* models of transdermal delivery, the skin barrier may be of differing ages, source, body site or species; it may be stored and pretreated in various ways and in some cases may even be replaced by synthetic membranes (e.g. membrane filters). Although these manipulations may aid in theoretical modeling (e.g. hydration of skin for 24 hours prior to study), these simplistic considerations of skin often underestimate its physiologic function *in vivo*. Comprehensive reviews of skin structure should be consulted (2,3).

Skin Anatomy as It Pertains to Barrier Function

The skin is a multilaminar organ which can be divided into the epidermis, dermis and hypodermis or subcutis. The dermis consists of dense irregular connective tissue made up of fibrous proteins (collagen, elastin, and reticulin) embedded in an amorphous hydrophilic ground substance. Branches of arteries, veins, nerves, and lymphatics run vertically from the dermis-subcutis junction to just beneath the basement membrane which separates the dermis from the epidermis. Adnexial appendages including sebaceous and sweat glands and hair follicles originate in the dermis and penetrate the surface. The dermis, whose predominant cell types include fibroblasts, mast cells, and macrophages, is thought to provide mechanical support, allow for the exchange of metabolites between blood and tissues, provide protection against infection and facilitate tissue repair. When topically applied molecules have penetrated through the epidermis and into the dermis, they are absorbed by the vasculature (unless specific binding occurs) which maintains a concentration gradient across the epidermis. Since one of the most important functions of mammalian skin is thermoregulation, and heat loss is controlled by dermal blood perfusion, the vascular component of the dermis is very well developed and regulated in homeothermic animals and humans.

The epidermis is adhered to the dermis by a multi-layered basement membrane. In addition to providing structural support, it acts as a semipermeable membrane for oxygen, nutrients and other molecules, although its role in preventing compounds from penetrating the skin is minimal. It is also the target of select chemical vesicants and is often involved in autoimmune diseases. The epidermis is a metabolically active structure. The stratum basale overlies the basement membrane and consists of a single layer of viable and nucleated cuboidal cells. Drug biotransformation by both phase I and II pathways occurs in these cells, however this process does not seem important in EATDD since there are no references to support extensive first-pass biotransformation after iontophoretci delivery. As these cells age, they differentiate and migrate into overlying layers. The cells of the upper stratum granulosum layer contain both non-membrane bound keratohyalin granules as well as lamellated bodies called membrane-coating granules. As these cells move superficially in the epidermis, the lamellated bodies fuse with the plasmalemma and extrude their lipid contents to contribute to the intercellular barrier matrix of the overlying stratum corneum. Pre-packaged cytokines may also be released from non-viable cells in the upper epidermal layers thereby playing an early role in modulating dermal irritation. The keratohyalin granules form the basis for the mass of the outermost stratum corneum which consists of stacks of flattened keratinized cells. The final product of epidermal differentiation is flattened, thick plasma membrane-limited cells containing fibrous keratin and keratohyalin, surrounded by an extracellular lipid matrix. This configuration has been described as protein "bricks" imbedded in a lipid "mortar" (4). It is this intercellular lipid matrix which is considered to be the primary barrier and paradoxically the pathway for transdermal drug delivery and remains the focus of research efforts to abrogate it.

The structure of the epidermal intercellular lipid matrix is complex (Figure 2). Intercellular spaces are narrow and are filled with alternating hydrophobic and hydrophilic regions (5). The hydrophobic regions in the stratum corneum consist of straight closely packed and almost entirely saturated hydrocarbon chains of lipids which contribute to its impermeability to most compounds including water. Lipids identified in the epidermis include phospholipids, ceramides, glycosylceramides, gangliosides, sterol esters, cholesterol, and fatty acids; and varies with skin depth. Polar lipids such as glycosphingolipids and phospholipids are present in basal layers while nonpolar lipids such as ceramides and cholesterol predominate in the stratum corneum (6). Recent work has attempted to modulate barrier function by altering epidermal lipid synthesis (7). Vehicles used in topical applications may alter epidermal lipid struc-

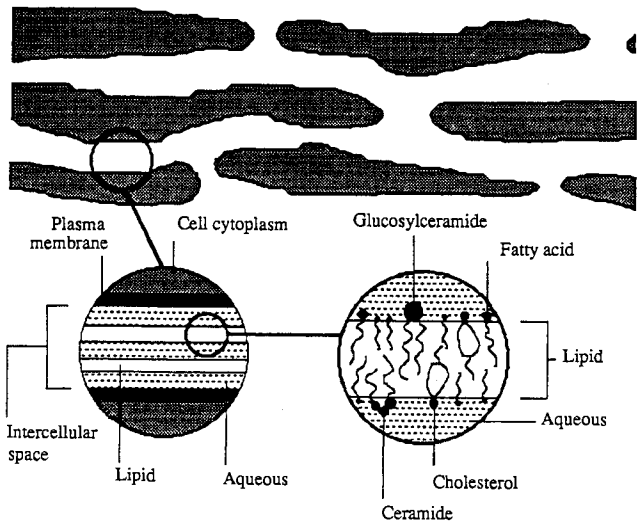

Fig. 2. Schematic representation of epidermal lipid structure.

ture thereby changing the skin's barrier function. With EATTD, these lipids must be traversed when drugs enter the skin via the intercellular route. Applied electrical fields also alter this lipid matrix which results in a decreased impedance with resulting increased chemical permeability. The mechanisms of these changes are being investigated.

Routes of Skin Penetration

For a compound to reach the systemic circulation there are three routes by which it can traverse the stratum corneum: 1) transappendageal (shunt); 2) intracellular (transcellular); and 3) intercellular (paracellular). The relative importance of each pathway many depend on may factors including the chemical characteristics of the penetrant such as solubility, partition coefficient, pK_a, molecular size, stability and binding. The composition, thickness, and source of the limiting membrane, including density of hair follicles and sweat glands, as well as its hydration status are important, albeit often forgotten factors.

Investigations which localize the route taken by ions as they cross the skin include experiments which attempt to visualize actual pathways or employ pharmacokinetic studies which provide indirect evidence for a particular route. Hair follicles and sweat glands have been postulated to provide a shunt pathway by which compounds can cross the skin without being exposed to the lipid environment of the stratum corneum (8). Early evidence using dyes suggested iontophoretic current traveled primarily through sweat glands and "skin pores" (9,10). A vibrating probe electrode with a spatial resolution of 5–40 μm identified appendageal pathways as carrying most of the current through hairless mouse skin *in vitro* (11). More recently (12,13) techniques which allow visualization of high conductance skin pathways during iontophoresis have been employed. Counter directional transport of $Fe(CN)_6^{4-}$ and Fe^{3+} across skin resulted in the precipitation of colloidal prussian blue, $Fe[Fe(CN)_6]_3$, which was recorded by video microscopy. Localized deposits of this compound were considered indicative of low resistance pathways. In a related method, flux of $Fe(CN)_6^{4-}$ was directly imaged by scanning electrochemical microscopy. These studies indicated iontophoretic flux was highly localized and the pathway dependent upon the structure of the skin used. The authors propose that this localized flux occurred through "shunt pathways" which are activated by the applied current. In skin with a high density of hair follicles (hairless mouse), appendageal transport prevailed. For a compound to enter the systemic circulation by any route including hair follicles, stratum corneum must still be penetrated. It is probable that the nature of the electrical resistance across the hair follicle may be different than that across non-haired skin because of the increased surface area of the stratum corneum lining the invagination relative to the topical area of the electrode application, coupled with the nature of the unique follicular blood supply. In contrast, sweat and sebaceous gland ducts are not lined by cornified epithelium and may provide a more accessable path for ion delivery.

Experimental evidence for inter- and intracellular pathways of drug flux has been reported. Iontophoretic delivery of $HgCl_2$ in excised human skin (14) and *in vivo* porcine skin (15) resulted in precipitate being localized by transmission electron microscopy to the intercellular matrix of the stratum corneum and deeper layers. In support of this, iontophoresis has been shown to increase the access of ions and water to the intercellular lipid lamellae (16).

The literature is not in agreement on the definitive route by which drugs are able to cross the skin during iontophoresis. The path of lowest electrical resistance may correspond to anatomical (e.g. hair follicles, sweat ducts) or low resistance intercellular (through polar regions of the lipid lamella) and intracellular pathways. Both human and porcine skin is characterized by a low density of hair follicles, potentially making extrapolation from other more haired species, problematic.Once into the stratum corneum, drug flux may branch to multiple pathways including appendageal, intracellular and intercellular routes, depending on resistance and the path of the electrical field. Local diffusion (including laterally) and tissue binding may modulate drug movement. Pathway issues have obvious toxicological significance since any adverse response seen will be dependent upon where the drug has passed or is accumulated.

Modeling

Nernst-Planck Theory

Many researchers have mathematically modeled iontophoretic drug flux. Interested readers should consult in-depth reviews (17,18). The Nernst-Planck equation describes membrane transport of ions under an electric field and has been used to describe iontophoretic transport (19–21). It is written as:

$$J_i = -D_i \frac{dc_i}{dx} - \frac{D_i z_i F c_i}{RT} \frac{d\phi}{dx} \quad (1)$$

where J_i is the flux of the *i*th species in a convection-free media, D_i is its diffusion coefficient, c_i its concentration and z_i its charge. *F* is the Faraday constant, R is the gas constant, T is the absolute temperature, and ϕ the electric potential. This equation implies that the total flux of an ionic species is equal to that flux due to a concentration gradient plus the flux caused by an electric field. When the species is uncharged ($z = 0$) or if no current is applied ($d\phi/dx = 0$), the Nernst-Plank equation reduces to:

$$J_i = -D \frac{dc_i}{dx} \quad (2)$$

or Fick's law of diffusion. On the other hand, if the concentrations of ions are equal on either side of the membrane ($dc_i/dx = 0$ or concentration is constant) then flux will be due to electrophoretic movements alone. In addition, as is likely with large hydrophilic or charged molecules, D may be so small as to minimize passive flux. The resulting equation:

$$J_i = -\frac{D_i z_i F c_i}{RT}\frac{d\phi}{dx} \quad (3)$$

adequately predicts the direct proportionality of iontophoretic flux to electric field strength. However, it provides no explanation for experimental results in which there was iontophoretic enhancement of uncharged compounds.

Convective Flux and Electroosmosis

Iontophoretic flux does not adequately predict all drug movement under an electric field. The transdermal flux of two nonelectrolytes (Ara-A and d-Thymidine) via anodal iontophoresis was more than four times greater than passive flux in *in vivo* mouse skin (22). Iontophoresis of thyrotropin releasing hormone (TRH) across nude mouse skin was studied under conditions which allowed it to be either neutral or highly protonated (23). TRH delivery was greater compared to passive delivery in either state. However contrary to predictions, higher flux was seen when the molecule was uncharged. To explain these results a third contribution to total flux can be added to yield:

$$J_{it} = J_{ie} + J_{ip} + J_{ic} \quad (4)$$

where J_{ie} is the flux of a drug due to electrophoretic movement (ionic flux), J_{ip} is passive flux, and J_{ic} is the flux of drug due to convective flow. The concept that a convective flux contributes to overall flux dates back to at least 1939 when electroosmosis was first studied (24). When an electrical potential is applied across a porous membrane with fixed charges, bulk fluid flows in the direction of counter-ion migration. The mechanism of this convective flow has been debated. Ions attracted to the fixed charges, under an applied potential, will migrate towards the electrode of opposite charge. This was proposed in 1924 (25) on the basis that since keratin's isoelectric point is between pH 3 to 4, at greater pH skin behaves as a porous membrane with fixed negative charges. During iontophoresis positive ions in the electrolyte solution are attracted to the pore walls and migrate from anode to cathode under an electric field. The movement of these counter-ions is thought to cause convective flow. This characteristic of skin has recently been termed permselectivity and allows skin to be functionally considered as similar to an ion exchange membrane.

A consequence of permselectivity can be seen in an iontophoretic cell containing equal concentrations of NaCl in the donor and receptor compartments. Although equal amounts of anodal driven current would be carried by the penetration of a Na^+ or the egress of a Cl^-, experimental results show that twice as much Na^+ flux occurs compared to that of Cl^- (26). Transport number (t_i) is defined as that proportion of the total current carried by a particular ion; $t_i = I_i/I$. In the preceding experiment, Na^+ was assigned a transport number of 0.6 and Cl^- 0.3 under the conditions tested with the remainder of the current being unaccounted for. Interestingly, Na^+ has a transport number of 0.4 in aqueous solution reinforcing the notion of the skin as a permselective membrane. In general, the most mobile positive ions have the highest transport number through a negatively charged pore and are preferentially delivered from an electrolyte solution. Thus, small ions such as Na^+ and H^+, which are often contained or generated in the donor compartment, can effectively compete with larger drug cations intended for anodal iontophoretic delivery. Even Cl-, although an anion, may be preferentially transported (from receptor to donor) if the drug ion is not particularly suited for iontophoretic delivery. This phenomenon is the single most important formulation variable which separates classic diffusion-driven passive delivery from electrical-based active delivery systems.

Phipps and Gyory (27) provide a detailed discussion of transport number and iontophoretic ion migration. Briefly, an iontophoretic system is considered as a donor compartment with an aqueous solution of drug ions and similarly charged inorganic co-ions, and a receptor compartment containing counterions, all of which compete to carry ionic current. A mathematical model is developed and experimental evidence provided illustrating a negative relationship between coion concentration and drug flux through a synthetic aqueous membrane or excised pig skin. This prompted the authors to propose that the iontophoretic delivery occurred through an aqueous channel in the skin. The importance of counter-ions in the receptor solution as competitors for carrying current is also stressed. Based upon an analysis of this model, the authors conclude that the "apparent" NaCl concentration of the epidermis is hypotonic at ~0.09 M and thus this concentration should be used in the receptor solution of *in vitro* studies. Since the reservoir in *in vivo* iontophoretic studies is viable skin, it is difficult to measure or control its ionic composition. This fact makes extrapolation of *in vitro* results to *in vivo* iontophoresis problematic, a conclusion which continues to be made in the literature (28).

Due to large relative molecular size and low ionic mobility, often the drug intended for transdermal delivery carries little current as compared to more mobile ions such as Na^+, H^+, and Cl^-. In one TRH study, <0.1% of the current was carried by TRH in its fully charged form during iontophoretic delivery (23). The preferential flux of smaller more mobile ions was seen to affect the iontophoretic flux of a series of amino acids and tripeptides. Cathodal iontophoresis of a negatively charged amino acid from a HEPES buffered saline solution achieved steady state flux after approximately five hours of current. In contrast, anodal iontophoresis of a positively charged amino acid resulted in continually increasing flux throughout the 18 h experiment, believed due to preferential transport of Na^+ followed by donor solution depletion. As Na^+ is transported, its concentration in the donor compartment decreases allowing more current to be carried by the positively charged amino acid penetrant (29).

Several mechanisms have been proposed for bulk fluid movement and associated movement of nonelectrolytes caused by counter-ion migration. Interestingly, if the donor and receptor solutions contained no ions, water molecules, with positive dipoles, migrate from anode to cathode resulting in water flow. This fluid movement was termed iontohydrokinesis and was most likely due to iontophoretic movement of ions whose dipoles attract a shell of hydration (22). Some have suggested it was due to an electrophoretic volume force and electroosmosis. An "ion atmosphere" of counterions forms lining the pore in the skin, and interaction with an applied electric

field produces the force necessary to move a volume of fluid towards the cathode. Additionally, movement of positive ions towards the cathode induces a concentration gradient which causes water to migrate (30,31). Convective flow may also be explained by non-equilibrium thermodynamics (32). In a system analogous to thermoelectric coupling, it is suggested that a pressure gradient and voltage gradient are conjugate forces in an iontophoretic delivery system. An applied voltage potential induces a pressure differential which directly causes fluid movements. Although the two may not be mechanistically identical, the terms electroosmotic and convective flux are often used interchangeably. This flux is:

$$J_{ic} = c_i v \quad (5)$$

where v is solvent velocity. This predicts convective flux to be proportional to concentration of the solute. In addition it has been shown that this flow is proportional to applied current (23). Taking this into account, total flux now becomes

$$J_{it} = J_{ie} + J_{ip} + J_{ic} = J_i = -D\frac{dc_i}{dx} - \frac{D_i z_i F c_i}{RT}\frac{d\phi}{dx} + c_i v \quad (6)$$

The contribution that each component makes to total flux depends on the physical properties of the penetrant including whether or not it is charged and of which sign. For anodal delivery of a cation, all flux components act synergistically. For cathodal delivery of an anion, electroosmosis counters ionic flow. Theory and some confirmatory experiments (33) also predicts electroosmotic contribution to overall iontophoretic flux to increase as molecular size increases. It is suggested that the delivery of a very large anion may be more effective via anodal iontophoresis and convective flow than by cathodal iontophoresis. In general however, as molecular weight increases, total flux decreases most likely due to decreasing diffusivity, steric hindrances and other interactions between the transported molecule and the purported trans-corneum channels. For small ions, ionic or electrophoretic flux will be most important whereas for neutral species, ionic flux is zero, thus electroosmotic flux dominates. When one considers mixed solutions of many different ions with different transport numbers, a complex situation often arises.

Although passive diffusion is considered insignificant in most circumstances, it has been shown that skin permeability to passive diffusion may increase with passage of iontophoretic current (34–37). Microscopically, an alteration in epidermal morphology has been described after lidocaine iontophoresis (38). Recent studies using transepidermal water loss and impedance spectroscopy at relatively low currents used in prototype clinical iontophoretic delivery systems (39) confirm that iontophoresis perturbs the barrier, a finding which complicates the strict application of electrochemical principles to the prediction of *in vivo* delivery since permeability is not constant.

These results suggest the existence of a potential energy barrier which can be modified by application of a potential difference (18). Structurally, this modification could involve the reorientation of molecules in the skin's lipid barrier similar to reversible dielectric breakdown seen in synthetic bilayers. If the skin is represented as a series of these potential barriers, then the sum of the potential drop across each interface equals the total voltage drop across the skin. Although the equation and derivations are not presented here (18), best fits to one model yielded 15 interfaces which is consistent with the number of cell layers of the stratum corneum. This result prompted the authors to suggest a nonappendageal route of transport for most ions, a finding in agreement with the $HgCl_2$ transport studies discussed above. However, this mechanism also begins to "blur" with that of strategies such as electroporation, which specifically apply higher voltages to cause impedance breakdown.

Electroporation

In contrast to the modes of EATDD discussed above which are primarily employed as electromotive forces to "drive" drug across the skin, electroporation may be employed to alter skin permeability such that resistance to drug transport is reduced. Electroporation is widely used today in cell culture systems to "insert" genes and other large molecules through cell membranes. The electric pulse produces a rapidly reversible polar pathway through lipid membranes which allows large molecules to pass. When the voltage is terminated, the membrane anneals and normal permeability characteristics are returned.

Electroporation has been employed in transdermal drug delivery by coupling it with iontophoresis (40–43). In these cases, a short (few milliseconds) pulse of high voltage alters the skin permeability such that subsequent iontophoresis is facilitated. Unlike electroporation of simple bilayer lipid membranes which anneal immediately, the complex lipid matrix of the stratum corneum has a slower return to normal permeability which permits enhanced iontophoretic delivery for longer (few minutes) periods of time. This is probably secondary to macromolecular interactions with the complex biochemical constituents seen in skin. Our laboratory studied the transdermal delivery of LHRH by electroporation and showed more rapid onset time and an enhanced flux over iontophoresis alone, and in some cases a reduced variability in the transdermal efflux profiles.

Closely related to electroporation is electroincorporation (44). This mode of EATDD is very similar to electroporation except particles (micropsheres, liposomes) are placed on the surface of skin and subsequent high voltage electrical pulses are employed. The current hypothesis as to the mechanism of action is that the spheres with the proper dielectric properties tend to concentrate the electric field at "focussed" regions at the contact of the sphere and skin which allows electroporation to occur at these points. Additionally, a second electromotive phenomenon occurs called dielectrophoresis which actually propells the particles along the electroporation-induced paths of lower impedance, through the skin. This phenomenon has not received as much attention as the more *classical* modes of electro-transport have and thus further discussion would be speculative.

Parameters for Characterizing Iontophoretic Delivery

The strides achieved by experimental and modeling studies have allowed identification of certain parameters and conditions that are important in iontophoretic delivery. These factors include physicochemical properties of the penetrant, donor and receptor solution composition, electronic factors, and barrier properties. In some cases, these parameters have predictable effects based on the equations presented, however others are less well defined and require further studies. Their extrapolation

to the *in vivo* setting may be problemmatic since in many cases, the *in vitro* systems used are artificial or utilize transport pathways significantly different than *in vivo* humans. For example, in an *in vitro* system, varying the thickness of dermis modulates the overall impedance such that the site of local lowest resistance (which dictates current pathway) may be different than if artificial sectioning did not occur. Similarly, edge effects where the diffusion cell is clamped together often result in a significant fraction of total current passing through this outer ring of damaged tissue. Similarly, various skin pre-treatments (prolonged soaking in water or saline) may hydrate the epidermis to a state not seen *in vivo*. These effects are often ignored yet may impact on interpretation of the results.

In Vitro *to* In Vivo *Correlations*

In our laboratory, the *in vitro* delivery of LHRH in the isolated perfused porcine skin flap (IPPSF) model has been shown to be predictive of its *in vivo* iontophoresis (45). The IPPSF has an intact and viable skin barrier and a functional intact microcirculation (46,47) Input data from the IPPSF flaps, treated as a "living" infusion pump, combined with the intravenous kinetic data (convolution) was observed to accurately predict the *in vivo* iontophoretic delivery (45). The immunoreactive LHRH delivered (as measured by radioimmunoassay) was also bioactive as LH and FSH concentrations were stimulated *in vivo*. This model has accurately predicted the *in vivo* human disposition of iontophoretically delivered arbutamine (48). This type of approach has been used for many types of *in vitro* systems as long as the rate limiting process is present in the model and quantitatively affects delivery the same as *in vivo*. In a study which attempted to evaluate the physicochemical basis for *in vitro-in vivo* correlations in iontophoretic delivery, a similar process of convolution was applied (49). The effect of an electrode design with a rate limiting membrane, chemical penetration enhancers and removal of the stratum corneum by tape stripping was investigated. Finally, when performing the convolution process, both process must be assumed to be independent and a boot-strap approach may be optimal to predict both the mean and variance of the *in vivo* concentration-time profile (50).

The most prudent approach for extrapolating from *in vitro* models is to test out any hypotheses with intact animals or preferably humans. This assures that the results for the specific drug of interest are valid and it builds up a database of when such extrapolations are not valid. Specific instances are alluded to in the discussions below.

Physicochemical

The magnitude of a compound's potential for iontophoretic delivery is theoretically proportional to its charge. The polarity of the charge will determine whether it should be delivered by anodal or cathodal iontophoresis. Isoelectric focusing and capillary zone electrophoresis has been used to screen compounds with respect to their potential for being delivered iontophoretically (51,52). A compound's molecular weight, calculated molar volume as well as solute radius have been shown to be inversely related to iontophoretic mobility (29,53).

A compound's hydrophobicity has been shown to have a negative influence on iontophoretic enhancement (54). Using a series of alkanoic acids of increasing chain length, the permeability coefficient for iontophoretic delivery decreased while that for passive delivery increased. Enhancement ratio, defined as iontophoretic flux divided by passive flux, showed an even stronger negative relationship to alkyl chain length. As expected, uncharged alkanols showed increased passive absorption with increasing hydrophobicity, however their iontophoretic flux (presumably via convective flow) increased when chain length was increased from 1–5, but decreased as chain length became greater than six. The authors suggest that these molecules were sufficiently hydrophobic to limit their ability to associate with water molecules which comprise convective bulk flow. In addition, since iontophoretic permeability was seen to decrease to below that of passive diffusion, the authors suggest that in this case iontophoresis may have a detrimental effect on the lipoidal pathway.

Wearley and co-workers (55) used a series of increasingly hydrophobic neutral amino acids to investigate the effect of binding on iontophoretic delivery. It was found that binding decreased with increasing hydrophobicity suggesting this binding is polar or electrostatic in nature. Binding was found to delay the onset of iontophoretic enhancement. The authors concluded that the hydration status of the skin had a significant impact on the iontophoretic permeation profile. Many *in vitro* models require extensive hydration of skin prior to transport studies, an intervention that could easily effect the mechanism of transport observed.

A recent study probed the effect of specific structural motifs on the EATD of LHRH (56). These studies showed that juxtaposition of cationic and hydrophobic amino acid residues in this oligopeptide significantly reduced electroosomosis and thus transdermal delivery of this peptide. It was proposed that the presence of both hydrophobic and cationic moities anchored the peptide within the skin and neutralized the normal negative charge of the skin pathway, thereby eliminating the skin's permselective property that facilitates electroosomotic flux. It is clear that as the mechanism of EATDD is further probed, special cases favored by select molecules and/or properties will further complicate the use of simple theoretical models based on parameters such as total charge or molecular weight, since what at first appear to be independent mechanisms of EATDD may ultimately be shown to be closely interdependent and resistant to a simple reductionist solution.

Donor and Receptor Solution Composition

The composition of donor and receptor solutions can greatly influence iontophoretic delivery. The receptor solution (e.g. the dermis *in vivo*) is obviously difficult to control yet in *in vitro* studies its effects on delivery may be profound. Three important considerations should be taken in formulating a donor solution: 1) drug concentration, 2) solution pH, and 3) buffer concentration. A direct relationship between drug concentration and iontophoretic flux was observed for cathodal acetate delivery (57) as well as anodal delivery of arginine-vasopressin (AVP) (58).

Donor solution pH has a complex influence on iontophoretic drug flux. Hydrogen and hydroxyl ions in the donor solution effectively compete to carry ionic current thereby reducing flux. It is beneficial to minimize their presence as well as other mobile ions. However, the charged character of proteins,

peptides and weak acids and bases is dependent upon pH according to the Henderson-Hasselbach equation. Additionally, in order to control pH changes induced by electrochemical reactions at the electrodes, buffering moieties must be included in the donor solution. If buffers are ionic and are able to compete with drug ions to carry current, efficiency will be reduced. Increasing NaCl concentrations in the donor solution has been shown to cause a decrease in benzoic acid flux through hairless mouse skin (59). A similar result was observed with a decrease in arginine-vasopressin permeation in response to increased buffer ionic strength. (58) The enhancement factor was seen to decrease approximately 10 fold as a result of an approximate 10 fold increase in ionic strength. A balance must be struck between drug concentration, solution pH, and buffer ionic strength in optimizing iontophoretic delivery. Strategies to eliminate the presence of mobile ions have been utilized.

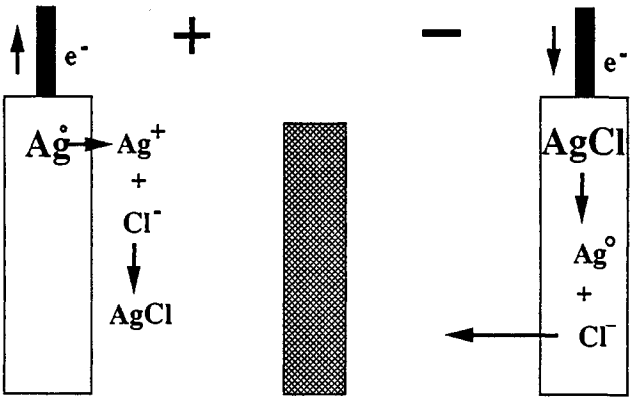

Fig. 3b. Electrode reactions with silver/silver chloride electrodes.

Electronic Factors

The electrodes of an iontophoretic cell are responsible for converting electronic current into ionic current. Electrons supplied to the cathode by the voltage source cause reduction while oxidation occurs at the anode. Submerged in an aqueous solution, the electrodes generate hydrogen, hydroxyl and hydronium ions (Figure 3a) which both alter the pH as well as compete with drug ions to carry current (60). Salt bridges and ion exchange membranes have been used in *in vitro* studies to separate ion generation from the donor solution. Although effective, these systems are impractical for *in vivo* iontophoresis. The use of silver/silver chloride electrodes has been widely accepted as an effective design for controlling electrolysis and pH of the electrode solutions (Figure 3b). Oxidation of silver wire at the anode releases Ag^+ which associates with Cl^- to precipitate as AgCl on the electrode. Reduction of AgCl at the cathode yields Ag and Cl^-. One potential limitation of this design is the necessary inclusion of Cl^-, usually as NaCl, in sufficient amounts to allow the formation of AgCl to continue. One mA-h of current generates 0.037 meq of Ag^+ thus equal amounts of Cl- must be present. The inclusion of Na^+ to supply Cl- ions could interfere with anodal iontophoresis. Similarly, generation of Cl- at the cathode might interfere with transport of negative drug ions or may provide increased counterions to interfere with anodal iontophoresis. Despite the benefits of Ag/AgCl electrodes, some researchers suggest they cause precipitation of peptides and protein drug intended for delivery and therefore employ carbon (57) or platinum wire (58) electrodes.

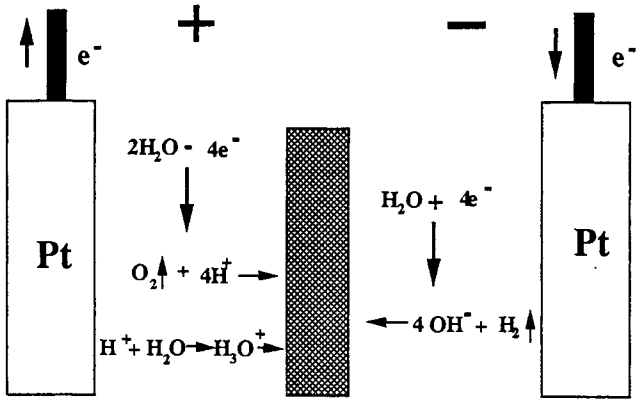

Fig. 3a. Electrode reactions with platinum electrodes.

A second concern of the electrode design is that of proper electrode contact such that ionic current is distributed over the entire electrode surface area. For most *in vitro* studies, complete contact between electrode and skin is ensured because the skin is immersed in the aqueous donor solution. In most cases the electrode conductive metal is surrounded by cotton, cloth or other porous material or gels which attempt to provide homogeneous contact with the skin's surface.

Iontophoretic current intensity has been shown to be directly proportional to iontophoretic flux (37,61,62). In most cases, a constant direct current (dc) is employed although other types of currents have been suggested. In attempts to minimize polarization of the stratum corneum, a pulsed dc current has been employed (63). The application of a direct current for a short period of time followed by a period of zero current is thought to allow the skin to recover and repolarize, therefore causing decreased skin resistance and greater iontophoretic delivery. In contrast to these findings, pulsed dc current at frequencies of 2-16 Hz allowed delivery of arginine-vasopressin but was no more effective than simple dc current (61). Pikal and Shah (64) postulated that pulsed dc current imparts an "impact energy" to the solvent which should allow greater glucose transport via convective flow. The optimal current strategy requires further study and may differ based on the drug intended for delivery.

Skin Barrier Factors

The replacement of the skin by synthetic membranes (e.g. stacked nucleopore, cation exchange membranes), although providing insight into the physicochemical nature of skin, discounts any biological influences (i.e. binding, metabolism) that may affect transdermal drug delivery. Since skin used for *in vitro* permeation studies must often be stored prior to its use, different protocols (i.e. freezing, thawing, hydration) have been suggested for best maintaining its integrity and relevance. Caution should be taken in extrapolating these results to *in vivo*.

Microanatomical and biochemical differences exist between species and therefore extrapolation between species is often problematic. Differing epidermal lipid composition may affect iontophoretic profiles especially of those compounds which may employ lipoidal pathways in traversing the skin.

As stressed earlier, hair follicle anatomy and density varies greatly between species. Iontophoretic transport was increased 61 and 12% for acetate and octanoate, respectively using haired rat skin as compared to nude rat skin (54). The authors attributed the larger difference for acetate to the increased availability of shunt pathways in haired skin. Octanoate, being more hydrophobic, would likely use lipoidal pathways and therefore be less affected by increased appendageal density.

Fate of a Dose

Although much work has been done on identifying factors responsible for optimizing drug delivery out of an electrode, the fate of this dose once it enters the skin is much less clearly understood. A drug molecule penetrating the skin is potentially subjected to several physicochemical processes, each of which can affect is ability to enter the systemic circulation. The ability to manipulate certain of these processes may allow enhanced systemic availability or conversely, for local or regional applications, the ability to target drug within the skin.

Although the metabolic activity of the skin is thought to be low, the potential for this type of loss should be assessed and avoided if possible. Chemical modifications to a peptide's structure are known to increase the molecule's resistance to some enzymatic degradative reactions. Several investigators have reported the formation of drug depot following iontophoretic delivery (55,65). The exact mechanism for this formation is unclear but has been postulated to be caused by drug precipitation in the skin, specific and non-specific binding of drug to receptors, proteins or other cell constituents as it passes through the skin, and loss of the molecule's charge due to changing pH of the skin (66). This latter phenomenon can be conceptualized as delivering a charged molecule through the cutaneous tissue which has an increasing pH gradient as a function of depth. This perspective could be viewed as an experiment in pH gradient isoelectric focussing. Permeation profiles for insulin and calcitonin suggested a reservoir effect as exhibited by increasing cumulative amounts of these penetrants for 36 h despite active iontophoresis being terminated between 2–4 h (67). A similar result was not seen for vasopressin prompting the authors to suggest that this molecule either does not form a depot, or that the depot formed is quickly desorbed from the skin. Verapamil has been shown to form a large depot in skin (68), possibly due to functional groups of the molecule interacting with the skin. In a different study, the formation of an insulin depot was theorized to be the result of physical entrapment of the insulin molecule as the monomer or polymer, charge interactions with the skin, and/or adsorption to tissue surfaces. In this study however, cumulative amounts of immunoreactive insulin did not continue to increase after the current was terminated whereas the radiolabel (I^{125}) did, suggesting degradation was occurring in the skin followed by desorption and delivery of insulin fragments.

In a similar study similar, the mechanisms causing the formation of a skin depot of a peptide as well as quantification of an iontophoretic dose was investigated in our laboratory (69). The iontophoretic delivery of I^{125} labeled LHRH allowed mass balance determinations of an iontophoretic dose. It was found that following a 3 h active period, approximately 1.4% of the dosed remained in the skin whereas 0.7% of the dose was delivered into the draining circulation. Attempts to deliver this depot using a vehicle-only electrode resulted in a minimal fraction of this depot to be desorbed. In contrast, an electrode containing LHRH placed over the skin depot delivered significantly greater amounts in a 3 h period, far greater than could be explained by mobilization of the underlying depot. This suggests that the second iontophoretic episode not only delivered a small amount of previously "loaded" LHRH, but was more efficient in delivering drug from the second electrode. This could be explained by initial iontophoresis saturating binding sites such that drug molecules from a second electrode are free to pass through the skin to access subepidermal blood vessels. Alternatively, the barrier could have been reduced by the initial episode although no consistent alteration in morphology were observed to suggest increased permeability to LHRH.

Enhancers

Although the selection of skin for *in vivo* studies is straight forward, pretreatment or co-treatment of the skin provides the ability to dramatically influence iontophoretic flux. Hydration of the skin by soaking or by occlusion is one form of pretreatment. Ethanol as a pretreatment allowed the iontophoretic delivery of two peptides (70). Ethanol was used to increase the delivery of a developmental dopamine antagonist CQA 206-191 (66). Aqueous CQA solubility was increased five fold by mixture with ethanol which allowed for a similar increase in delivery from the donor side. However, this resulted in only moderate increases in transdermal transport due to a five fold increase in the amount of CQA that could be extracted from skin. Two explanations of this retention in the skin were pH changes in the skin converted a portion of CQA to its unionized form and therefore was no longer influenced by an electric field, or precipitation in the skin as a chloride salt. Ethanol as a pretreatment did not alter the flux of piroxicam unless oleic acid (OA), a fatty acid enhancer, was included and then it showed current dependence (71). The authors postulate that at low current densities the negatively charged OA ion competes to carry current with piroxicam thereby decreasing piroxicam flux. At higher currents, enough OA is able to enter the skin iontophoretically to allow it to act as a lipid enhancer thereby enhancing piroxicam delivery.

A second mechanism whereby co-iontophoresis of a compound may enhance drug delivery was demonstrated in our laboratory where co-iontophoresis of vasomodulating substances can alter drug flux (72). Co-iontophoresis of epinephrine, a vasoconstrictor, decreased lidocaine flux. Co-iontophoresis of tolazoline, a vasodilator, increased lidocaine delivery in the isolated perfused porcine skin flap (IPPSF) while decreasing its flux in *in vitro* diffusion cell studies (due to competition for transport). Unlike the *in vitro* model systems, the IPPSF maintains a viable microvasculature which is responsive to vasomodulation and thus is capable of detecting this effect. However, these effects are complex because vasomodulation may actually be changing the volume of the dermis being perfused, analagous in Figure One to dynamically altering the volume of the receptor cell (73).

Formulation and Iontophoretic Device

Iontophoretic delivery presents the drug formulator with several obstacles not encountered with conventional drug prod-

ucts, including stability of the drug under an electric field and the mobility of formulation components which decrease drug delivery efficiency. The potential electrolytic degradation of a compound or its degradation due to ionic interactions in the electrode must be investigated, in addition to the routine stability testing of drug substance and drug product. Nefopam was shown to be more stable when iontophoresed using silver/silver chloride electrodes compared to platinum electrodes (74). The authors concluded that hydrolysis of water caused by reactions at the platinum electrode (see before) caused a microenvironmental pH change resulting in decreased stability. In contrast, platinum electrodes were selected to iontophoretically deliver insulin because they are thought to be relatively inert (75). In this case, Ag/AgCl electrodes could not be used because they caused precipitation of the insulin.

Drug in solution is not readily amenable to incorporation into an iontophoretic device. To circumvent this, gel formulations have been investigated. The systematic investigation of the optimal physical properties for hydrogel-based iontophoretic devices was reported (67). The delivery of three peptides from formulation made with three types of gels were studied along with the gels' swelling, drug loading, and drug release characteristics. Gel viscosity is another parameter that can affect iontophoretic delivery since Waldens' Rule states that the product of viscosity and molar conductance is a constant. Therefore, an increase in viscosity should lead to a decrease in ionic mobility and iontophoretic permeability. Such an inverse relationship between gel viscosity and nefopam permeability coefficient was demonstrated (74).

DISCUSSION

This necessarily brief overview of electrically-assisted transdermal delivery hopefully has provided the reader with some basic concepts that are involved in active transdermal delivery and contrasts with the principles of diffusion-driven passive delivery. These approaches offer many advantages over oral and parenteral routes of administration (76).

It is evident that many factors can directly affect the ability to successfully deliver a particular compound using iontophoresis. Optimization of an iontophoretic delivery system may require a large commitment to basic research prior to taking these efforts into the clinics. The transdermal iontophoretic delivery of enalaprilat and cromolyn sodium was optimized by systematically investigating the effect of ionic strength, buffer type and size, drug concentration and pH (77). In each case the result of varying one of these parameter obeyed the previously described predicted relationships to iontophoretic flux. Factors which could affect the transdermal delivery of nefopam hydrochloride included drug concentration, pH, ionic strength and viscosity of donor solution, electrode type, and current intensity and voltage (74). Optimal conditions were selected not only to maximize iontophoretic flux, but also to minimize potential degradation of the molecule caused by iontophoresis.

This is an active area of research and development which suggests that the open scientific literature may not be the only source of information for addressing some of these mechanistic issues. This mode of delivery may be the best available for many of the relatively hydrophilic drugs of biotechnology (peptides, oligonucleotides) and thus warrants further study.

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