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**In Vitro Optimization of Dexamethasone Phosphate Delivery by Iontophoresis**

Jean-Philippe Sylvestre, Richard H Guy, M Begoña Delgado-Charro

**Background and Purpose.** This study was designed to evaluate the effects of competing ions and electroosmosis on the transdermal iontophoresis of dexamethasone phosphate (Dex-Phos) and to identify the optimal conditions for its delivery.

**Methods.** The experiments were performed using pig skin, in side-by-side diffusion cells (0.78 cm²), passing a constant current of 0.3 mA via Ag-AgCl electrodes. Dex-Phos transport was quantified for donor solutions (anodal and cathodal) containing different drug concentrations, with and without background electrolyte. Electrotransport of co-ion, citrate, and counterions Na⁺ and K⁺ also was quantified. The contribution of electroosmosis was evaluated by measuring the transport of the neutral marker (mannitol).

**Results.** Electromigration was the dominant mechanism of drug iontophoresis, and reduction in electroosmotic flow directed against the cathodic delivery of Dex-Phos did not improve drug delivery. The Dex-Phos flux from the cathode was found to be optimal (transport number of ~0.012) when background electrolyte was excluded from the formulation. In this case, transport of the drug is limited principally by the competition with counterions (mainly Na⁺ with a transport number of ~0.8) and the mobility of the drug in the membrane.

**Discussion and Conclusion.** Dex-Phos must be delivered from the cathode and formulated rationally, excluding mobile co-anions, to achieve optimal iontophoretic delivery.

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The use of corticosteroids for the treatment of a variety of musculoskeletal conditions is common practice. Local administration of the drug by injection into the relevant subcutaneous compartment usually results in improved clinical outcome, at least temporarily. Furthermore, when depot formulations are injected intramuscularly, the systemic effects associated with oral delivery are reduced. However, although injections are generally safe, they present some disadvantages and risks (eg, postinjection pain and flare, infection, difficulty in accurately placing the needle, need for an experienced health care professional to perform the injection).2,5

Alternatively, iontophoresis also is used in clinical practice to deliver corticosteroids locally. Iontophoresis is a minimally invasive technique that enhances the transport of charged and highly polar molecules across the skin by the application of a small electrical current (with a current density <0.5 mA/cm²). The 2 main mechanisms of transport of this electrically enhanced method are electromigration and electroosmosis.

Electromigration originates from the direct interaction of the electrical field and the ions present in the formulation and the skin, and therefore, will enhance the transport of cationic drugs from the anode and, inversely, negatively charged drugs from the cathode. Conservation of charge requires that the sum of the electrical current carried by each ion equal the total electrical current supplied by the power source. Practically, as far as drug delivery is concerned, this means that the drug competes with all the other ions present in the system. The efficiency of transport, that is, the fraction of the total charge transported by a given drug (its transport number, tₜₐ), can be determined experimentally by measuring its flux (Jₜₐ) and applying the relation:

\[ J \cdot t \cdot z \]

where I is the total current passed, F is the Faraday’s constant, and z is the valence of the drug.

Electroosmosis has its origin in the fact that the skin is a negatively charged membrane at physiological pH. When an electrical potential is applied across a membrane containing a fixed charge, a bulk volume flow of solution occurs in the direction of the counterion movement. This means that for the negatively charged skin, the electroosmotic flow is in the anode-to-cathode direction. This assists the transport of cations and retards that of anions. This flow of solvent carries through the skin any dissolved solute and, therefore, is the mechanism enhancing transdermal delivery of neutral, polar molecules. Electroosmotic flow is the dominant mechanism of transport for the delivery of larger molecules. The drug flux due to the electroosmotic mechanism \( J \cdot z \) is proportional to the concentration \( C \) of the solute:

\[ J = v \cdot C \]

where \( v \) is the solvent volume flow.

The formulation’s pH and ionic strength are the main parameters that can be used to modulate electroosmosis by, respectively, modifying and screening the skin’s charge. Dexamethasone phosphate (Dex-Phos, MW: 472.4), a phosphorylated prodrug of dexamethasone (Dex), undoubtedly has been the most studied corticosteroid for iontophoretic delivery. The disodium salt, dexamethasone sodium phosphate (Dex-Na₂ Phos, MW: 516.4), is water soluble and at physiological pH is present mainly in its dianionic form (pKₙₐₚ: 1.9 and 6.4). Iontophoretic treatment of various musculoskeletal problems with Dex-Phos has been the subject of clinical studies, many of which have reported beneficial effects. However, improvement in the patient’s condition is not always seen, perhaps due to the variety of iontophoretic conditions used and to the fact that the optimal conditions for Dex-Phos delivery have not been identified.

The delivery electrode and the formulation of the drug solution are controllable parameters that are expected to greatly influence the efficiency of the delivery. Earlier studies have used the anode to deliver Dex-Phos, but, given that Dex-Phos is negatively charged at physiological pH (z = 2 at pH 7.4), delivery from the cathode (with electromigration as the main driving force) is more appropriate than that from the anode (electroosmosis being the only available driving force). Furthermore, many clinical studies evaluating the iontophoretic delivery of Dex-Phos have combined the drug with lidocaine hydrochloride. Again, this practice may have a detrimental effect on Dex-Phos delivery due to the introduction of exogenous ions that are competing with the drug for the transport of current through the skin. Thus, there is a need to optimize the conditions for Dex-Phos iontophoresis.

The primary objective of this research, therefore, was to identify the optimal iontophoretic conditions to deliver Dex-Phos. A further goal was to better understand the effects of: (1) the ions present in the formulation or in the subdermal compartment that are competing with Dex-Phos to transport the current and (2) the electroosmotic flow, which normally occurs in the anode-to-cathode direction, that is, against the electromigration of Dex-Phos delivered from the cathode.
**In Vitro Optimization of Dexamethasone Phosphate Delivery by Iontophoresis**

**Method**

**Materials**

Dex (>98%) and Dex-Na2-Phos (>98%) were purchased from Sigma-Aldrich. Solutions of injectable Dex-Na2-Phos were obtained from American Regent (equivalent to 4 mg/mL Dex-Phos), Faulding (again equivalent to 4 mg/mL Dex-Phos), and Organon Laboratories (equivalent to 4.6 mg/mL of Dex-Phos). Topical lidocaine hydrochloride 4% (Xylocaine Topical 4%) was obtained from AstraZeneca. Sodium chloride, potassium chloride, sodium phosphate (monobasic), potassium phosphate (dibasic), mannitol, phosphoric acid (85%), and methanesulfonic acid (99%) were obtained from Acros Organics. Potassium citrate, methanol (high-performance liquid chromatography grade), acetonitrile (far UV high-performance liquid chromatography grade), acetate, methanol (high-performance liquid chromatography grade), ethyl acetate, and hydrochloric acid 37% (weight-to-weight ratio) were purchased from Sigma-Aldrich. All reagents were at least analytical purity and were used as received from the supplier. Potassium citric acid and 37% weight-to-weight ratio HCl were used to adjust the pH. Potassium citrate and water (trace amounts of extraneous anions inevitable when a strong acid is used to lower the pH of a 10 mM phosphate) at pH 7.4, an equimolar mixture of the diacid (dexamethasone-dihydrogenphosphate [Dex-H2-Phos]) and its conjugate base (Dex-Na2-Phos) was dissolved in water to obtain a final concentration of 0.4% of Dex-Phos. Dex-H2-Phos was prepared from the disodium salt by adding one part of 37% weight-to-weight ratio HCl to 3 parts of a solution of 25 mM of Dex-Na2-Phos in water. The insoluble dihydrogen acid precipitated and was recovered by liquid extraction using ethyl acetate. The ethyl acetate was evaporated, leaving a powder of Dex-H2-Phos, which contained only trace amounts of extraneous ions when assayed by ion chromatography.

**Iontophoresis**

In vitro iontophoresis experiments were performed in side-by-side diffusion cells, as illustrated in Figure 1 (transport area = 0.78 cm²). The skin was clamped between the 2 half cells, with the stratum corneum side facing the donor (drug-containing solution) chamber. In all experiments, the subdermal compartment was filled with phosphate-buffered saline (PBS; 170 mM sodium, 1.4 mM potassium, 137 mM chloride, and 18 mM phosphate) at pH 7.4. Both chambers held 3.5 mL of solution and were magnetically stirred. Thirty minutes prior to iontophoresis, the cathodal chamber was filled with 3.5 mL of deionized water and the anodal chamber was filled with 3.5 mL of deionized water (18.2 MΩ cm, Barnstead Nanopure Diamond®). The concentrations of Dex-Na2-Phos solutions are expressed in terms of the Dex-Phos concentration. For example, a 0.4% Dex-Phos solution contains 4.4 mg/mL of Dex-Na2-Phos.

**Skin Preparation**

Porcine ears were obtained from a local abattoir. Ears were cleaned under running cold water, and the skin was dermatomed to a nominal thickness of 750 μm (Zimmer Electric Dermatome®). The pieces of tissue obtained (~9 cm²) were wrapped individually in Parafilm and stored for no more than 3 months at −20°C until use. The required pieces of skin were thawed at room temperature for approximately 30 minutes before each iontophoresis experiment.

**Preparation of Dex-Phos Solution at pH 4**

In one experiment, a solution of Dex-Phos prepared in deionized water at pH 4 was used. To avoid the inclusion of extraneous anions inevitable when a strong acid is used to lower the pH of a Dex-Na2-Phos solution prepared in water (typical pH = 7.5), an equimolar mixture of the diacid (dexamethasone-dihydrogenphosphate [Dex-H2-Phos]) and its conjugate base (Dex-Na2-Phos) was dissolved in water to obtain a final concentration of 0.4% of Dex-Phos. Dex-H2-Phos was prepared from the disodium salt by adding one part of 37% weight-to-weight ratio HCl to 3 parts of a solution of 25 mM of Dex-Na2-Phos in water. The insoluble dihydrogen acid precipitated and was recovered by liquid extraction using ethyl acetate. The ethyl acetate was evaporated, leaving a powder of Dex-H2-Phos, which contained only trace amounts of extraneous ions when assayed by ion chromatography.

**Figure 1.**

Schematic diagram of the experimental setup.
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Table 1.
Effect of Donor Solution Composition on Dexamethasone Phosphate (Dex-Phos) Fluxes and the Derived Transport Numbers During Cathodal Iontophoresis

<table>
<thead>
<tr>
<th>Donor Solution</th>
<th>Competing Anions</th>
<th>Dex-Phos Flux (nmol/h), $\bar{X}$ (SD)</th>
<th>$100 \times$ Transport No., $\bar{X}$ (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injectable Dex-Phos 0.4% (Faulding)</td>
<td>Cl$^-$ (~100 mM)$^a$</td>
<td>2.1 (0.6)</td>
<td>0.04 (0.01)</td>
</tr>
<tr>
<td>Lidocaine 4% (AstraZeneca) (1:2)</td>
<td>Citrate$^{3-}$ (~13 mM)$^b$</td>
<td>8.8 (3.9)</td>
<td>0.16 (0.07)</td>
</tr>
<tr>
<td>Injectable Dex-Phos 0.4% (American Regent)</td>
<td>Citrate$^{3-}$ (~40 mM)$^b$</td>
<td>7.5 (2.9)</td>
<td>0.13 (0.05)</td>
</tr>
<tr>
<td>Injectable Dex-Phos 0.4% (Faulding)</td>
<td>Citrate$^{3-}$ (40 mM)</td>
<td>10 (5)</td>
<td>0.18 (0.08)</td>
</tr>
<tr>
<td>Dex-Phos 0.4% in potassium citrate</td>
<td>Citrate$^{3-}$ (40 mM)</td>
<td>8.8 (3.9)</td>
<td>0.16 (0.07)</td>
</tr>
<tr>
<td>Dex-Phos 0.4% in 0.9% NaCl</td>
<td>Cl$^-$ (154 mM)</td>
<td>14 (4)</td>
<td>0.25 (0.07)</td>
</tr>
<tr>
<td>Injectable Dex-Phos 0.5% (Organon)</td>
<td>None (glycerol for isotonicity)</td>
<td>81 (39)</td>
<td>1.4 (0.7)</td>
</tr>
<tr>
<td>Dex-Phos 0.4% in H$_2$O</td>
<td>None</td>
<td>69 (24)</td>
<td>1.2 (0.4)</td>
</tr>
</tbody>
</table>

$^a$ Deduced from the molar concentration of lidocaine hydrochloride in the final solution.
$^b$ Determined by direct measurement with ion chromatography.

Table 2.
Mannitol Flux in the Anode-to-Cathode Direction and the Corresponding Solvent Flow for the Experimental Dexamethasone Phosphate (Dex-Phos) Delivery Conditions Tested

<table>
<thead>
<tr>
<th>Dex-Phos Delivery Electrode</th>
<th>Donor Solution</th>
<th>Mannitol Flux (nmol/h), $\bar{X}$ (SD)</th>
<th>Solvent Flow ($\mu$L/h), $\bar{X}$ (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathode</td>
<td>Dex-Phos 0.4% in H$_2$O</td>
<td>52 (10)</td>
<td>5.2 (1.0)</td>
</tr>
<tr>
<td>Cathode</td>
<td>Dex-Phos 0.4% in 40 mM of potassium citrate</td>
<td>40 (6)</td>
<td>4.0 (0.6)</td>
</tr>
<tr>
<td>Cathode</td>
<td>Dex-Phos 0.4% in 0.9% NaCl</td>
<td>24 (4)</td>
<td>2.4 (0.4)</td>
</tr>
<tr>
<td>Anode</td>
<td>Dex-Phos 0.4% in 0.9% NaCl</td>
<td>32 (9)</td>
<td>3.2 (0.9)</td>
</tr>
</tbody>
</table>

of PBS to check for leaks. Both chambers then were emptied and refilled with the appropriate donor and receptor solutions before the experiment was started. In all experiments, a 0.3-mA constant current intensity (current density = 0.38 mA/cm$^2$) was applied for 6 hours via Ag-AgCl electrodes connected to a power source (Yokogawa 7651 Programmable DC source). All experiments were performed at room temperature with a minimum of 4 replicates using the skin from at least 2 different pigs. To measure the delivery of Dex-Phos, 1 mL of the subdermal compartment was collected and replaced by the same volume of PBS every hour for the first 2 hours and every half hour thereafter. A 24-hour control experiment, with no electrical current applied, also was performed using Dex-Phos 0.4% in water as the donor solution.

Effect of delivery electrode polarity. First, the effect of the polarity of the delivery electrode was verified for 2 formulations containing sufficient Cl$^-$ to satisfy the electrochemistry at the anode: (1) Dex-Phos 0.4% in 0.9% NaCl and (2) a mixture containing one part of injectable Dex-Phos (Faulding) for 2 parts of lidocaine hydrochloride.

Effect of donor composition. Dex-Phos delivery from the cathode was measured for a variety of donor solutions (Tab. 1). The effect of the concentration of the drug in the absence of a background electrolyte was verified for concentrations ranging from 0.2% to 0.8%. In one experiment, to ensure that the concentration of the Cl$^-$ released by the Ag-AgCl electrode remained an order of magnitude lower than that of the drug in the donor solution (Dex-Phos 0.4% in H$_2$O), the latter was continuously perfused (15 mL/h), using a peristaltic pump, during the iontophoretic delivery.

Effect of electroosmosis. A strategy to reduce electroosmosis against the delivery of Dex-Phos was explored. The approach involved delivery of 0.4% Dex-Phos from a donor solution at pH 4. In the latter iontophoretic conditions and those described in Table 2, electroosmotic flow was assessed using mannitol (at 10 mM) as a marker. The flux of mannitol was evaluated in both anode-to-cathode and cathode-to-anode directions. In addition to sampling of the receptor chamber, 0.3 mL of the

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*Yokogawa Measurement Technologies Ltd, Solar House, Mercury Park, Wycomber Lane, Woodburn Green, Bucks, HP10 0HH, United Kingdom.*
donor solution was collected hourly. The passive diffusion of mannitol across the skin also was measured as a control.

Contribution of competing ions. Finally, the donor solution samples obtained in the experiment with mannitol, in the 3 cases described in Table 4, were assayed for the major competing counterions sodium and potassium. The concentration of the co-ion citrate in the receptor also was measured when Dex-Phos was formulated with this anion. From these values, the contributions of the main competing ions to the total current were determined.

Sample Analysis
The concentrations of Dex-Phos and Dex in the receptor chamber were assayed by high-performance liquid chromatography (ASI-100 automated sample injector, P680 pump, TCC-100 thermostated column compartment, PDA-100 diode array detector, Chromeleon software)** under isocratic conditions. A mobile phase consisting of 30:70 (volume-to-volume ratio) acetonitrile:phosphate buffer (0.15 M, pH 2) was pumped (0.75 mL/min) through a Lichrosphere 100 RP-18 (4 × 125 mm) reverse-phase column*** fitted with its guard column and thermostated at 25°C. Dex and Dex-Phos concentrations were quantified via their UV absorbance at 240 nm using their respective calibration curves obtained from a minimum of 5 standard solutions (made from 500-ppm stock solutions in methanol appropriately diluted in PBS at pH 7.4) covering the entire range of experimental concentrations. The retention times for Dex-Phos and Dex were ~3.5 and ~10.5 minutes, respectively; the detection limit for both was 0.02 µg/mL for a 25-µL injection, and detection was linear up to 150 µg/mL (correlation coefficient >.999, relative standard deviation <5%).

Ion chromatography with suppressed conductivity detection (AS50 autosampler and thermal compartment, GP50 gradient pump, ED50 electrochemical detector)** was used to measure the concentrations of sodium, potassium, and citrate. For the quantification of cations, the 20 mM of methanesulfonic acid eluent was pumped under isocratic conditions (1 mL/min) through an IonPac CS12A column** (250 × 4 mm) thermostated at 30°C and a CSRS ULTRA II suppressor** (4 mm) set at a current of 62 mA. Concentrations of Na⁺ and K⁺ were measured against linear calibration curves obtained from standard solutions (at least 5 different concentrations, correlation coefficient >.998, relative standard deviation <6%) of their respective chloride salt. For the quantification of citrate anions in the receptor compartment, the 35 mM of NaOH mobile phase was pumped under isocratic conditions (1 mL/min) through an IonPac AS16 column** (250 × 4 mm) thermostated at 30°C and an ASRS ULTRA II suppressor** (4 mm) set at a current of 90 mA. At least 5 potassium citrate solutions were used as standards to generate the linear calibration curve (correlation coefficient >.999, relative standard deviation <4%).

Analysis of mannitol was performed using ion chromatography coupled with pulsed amperometric detection.** The mobile phase (380 mM of NaOH) was pumped (0.4 mL/min) through a CarboPac MA1 column** (250 × 4 mm) maintained at a temperature of 20°C. Again, the linear calibration curve was obtained from at least 5 standards of mannitol in PBS (correlation coefficient >.999, relative standard deviation <7%).

Data Analysis
Linear regressions and statistical analyses were performed using GraphPad Prism V.4.00.††† Statistical differences within multiple data sets were assessed by one-way analysis of variance, followed by a Tukey multiple comparison test. The level of statistical significance was fixed at P<.05. The fluxes were obtained from the slope of the cumulative amount delivered as a function of time for each replicate and are expressed as mean (SD). The reported Dex-Phos flux is the sum of the Dex-Phos and Dex fluxes into the receptor phase to account for the partial dephosphorylation of the prodrug that occurred during transdermal passage or in the receptor compartment. Transport numbers were calculated using these fluxes and equation 1. The valence used for Dex-Phos in the calculations was 2, unless otherwise stated.

Results
Passive Diffusion Control
The passive diffusion flux of Dex-Phos from a 0.4% solution in pure water after 6 hours was below the detection limit and reached only 0.15 (0.11) nmol/h at 24 hours. Considering that all iontophoresis experiments lasted 6 hours, the contribution of passive diffusion could be assumed to be negligible compared with electrotransport.

Anodic Versus Cathodic Delivery
Dex-Phos delivery from the anode was compared directly with that from the cathode. Two donor solutions, which contained sufficient Cl⁻ to ensure good functioning of the Ag-AgCl electrode during anodic delivery, were tested: (1) 0.4% drug in normal saline and (2) a mixture of commercially available citrate-buffered injectable Dex-Phos (Faulding, 0.4% weight-to-volume ratio) and lidocaine hydrochloride (4%...
weight-to-volume ratio). In the former case, cathodal and anodal fluxes were 14 (4) and 0.3 (0.2) nmol/h, respectively; for the latter, the corresponding transport rates were 2.1 (0.6) nmol/h from the cathode and below the level of detection from the anode.

Effect of Donor Composition on Cathodic Delivery

The next logical step was to optimize delivery from the cathode. In the case of the negatively charged Dex-Phos, the efficiency of iontophoretic delivery will be reduced by the presence of competing anions in the donor solution.11 Dex-Phos flux, therefore, was measured from different formulations, many of which had been used in clinical studies, containing a more or less constant concentration of the drug together with a range of background electrolytes. The Dex-Phos solutions tested, the drug’s flux, and the corresponding transport numbers are reported in Table 1. The pH of all solutions tested, except the mixture of Dex-Phos and lidocaine, was between 7.2 and 7.9, meaning that >85% of the drug had a charge of −2. However, because in all cases the majority of the drug was doubly charged, the value for z in equation 1, which was used to determine the transport number from the flux, was assumed equal to 2.

Clearly, the absence of background electrolyte (Dex-Phos in pure water and the injectable Dex-Phos formulation from Organon Laboratories containing a neutral molecule, glycerol, for isotonicity) greatly improved drug delivery. These 2 solutions resulted in fluxes that were not significantly different.

Effect of Electroosmosis

To evaluate the effect of electroosmosis on the delivery of Dex-Phos, the flux of mannitol in the anode-to-cathode direction and its equivalent solvent flow were measured for the 4 delivery conditions summarized in Table 2. The mannitol fluxes also were measured in the cathode-to-anode direction and were not significantly different from the negligible passive diffusion of the neutral marker (1.0 ± 0.6 nmol/h). Electroosmosis occurred against Dex-Phos delivery from the cathode. Solvent flow was maximal when no background electrolyte was present in the donor (Dex-Phos 0.4% in H2O), the condition so far identified as “optimal” for Dex-Phos delivery. Electroosmotic flow was significantly higher when the background electrolyte was potassium citrate compared with sodium chloride (P<.05). The presence of Dex-Phos in the anode, as opposed to the cathode, when normal saline acted as background electrolyte, did not signifi-
cantly affect the level of electroosmotic flow.

A strategy then was explored to reduce (or reverse) the skin’s negative charge in order to decrease the electroosmotic flow and thereby enhance the delivery of the drug. The Dex-Phos 0.4% in H2O solution was prepared at pH 4 using a mixture of the diacid (Dex-H2-Phos) and the disodium salt (Dex-Na2-Phos). The idea was to reduce the negative charge on the skin, the isoelectric point of which has been shown to be between 4 and 5.33 As shown in Table 3, mannitol delivery was clearly reduced at pH 4, and convective solvent flow decreased, but this had no impact on the electrotransport of Dex-Phos.

**Transport Numbers of Main Ionic Species**

Finally, the transport numbers of the main ionic species present in the different experimental conditions studied were measured (Tab. 4). It was clear that sodium was the major charge carrier across the skin, contributing more than 70% in all cases. Only when the pH was reduced to 4 was the Na+ transport lowered ($P<.05$). In addition, although all donor solutions eliminated Cl−, the Ag-AgCl electrochemistry resulted in the progressive release of this anion into the donor solution such that a significant contribution to the movement of charge could be deduced for this anion.

**Discussion and Conclusions**

**Electroosmosis**

Dex-Phos delivery from the cathode proceeded against electroosmotic flow. The latter increased when the background electrolyte was removed from the drug solution in good agreement with previous reports.15,35

Lowering the pH of the Dex-Phos donor solution to 4 reduced the electroosmotic flow in the anode-to-cathode direction as previously observed.15,33 Consistent with the reduction of electroosmotic flow, the transport number of Na+ was significantly smaller as well, reflecting a change in the skin’s permselectivity. However, the lower pH in the donor solution failed to improve drug delivery, suggesting that the altered electroosmotic flow has a negligible effect on the electrotransport of the drug.

Dex-Phos delivery from the anode was really inefficient and, therefore, should be avoided in the clinic. This conclusion contradicts an early study in which the drug was iontophoresed in vivo, in a monkey, from the anode and resulted in measurable levels in deep underlying structures.30 Although the exact reasons for this discrepancy are still unclear, 2 comments on the latter study can be made in that respect: (1) a relatively important drug concentration (>2 μg/g) in subdermal structures (depth >3 mm) was observed after a short application of the drug (20 minutes) in absence of current, which raises the question whether the skin barrier in this animal model was compromised; and (2) the current density used (0.94 mA/cm²) is nearly twice the maximum tolerable level in humans (0.5 mA/cm²)34 and could have altered the skin barrier.

**Ionic Competition**

Not all commercially available Dex-Phos solutions are equivalent for iontophoresis. The ideal donor solution contains no background electrolyte to compete with the drug to carry a charge across the skin. Trends similar to those observed in this study had been observed, but to a lesser extent due to a much higher passive diffusion contribution, when Dex-Phos was iontophoresed across an artificial membrane.35 The Dex-Phos/lidocaine formulation resulted in a Dex-Phos flux much less than those from all other formulations due to the high levels of chloride and citrate present. Although lidocaine hydrochloride increases the buffer...
capacity of the donor solution, this is not necessary when Ag-AgCl electrodes are used because problems associated with pH changes are avoided. In fact, in the experiments reported here, the pH of the donor solution never varied by more than 0.2 pH unit over the 6 hours of iontophoresis.

Even in optimal delivery conditions, the transport number of Dex-Phos was relatively small, with values just over 0.01. This was most likely due to the rather poor mobility of the drug inside the membrane resulting from its relatively large molecular weight. Similarly, the transport numbers of cations were found to fall off rapidly with molecular weight, with values approaching zero at 400 to 500 Da. The principal ionic species carrying electrical current during delivery of Dex-Phos from water was the subdermal counterion Na⁺. Even when citrate was present, the transport number of sodium was >80%. This value is in good agreement with the transport number of sodium previously observed when the competing counterion was gluconate, an anion with a molecular weight very similar to that of citrate. Interestingly, the release of Cl⁻ from the Ag-AgCl cathode during iontophoresis of Dex-Phos from water did not significantly affect drug transport over the course of the experiment. Given the amount of Cl⁻ produced (~67 μmol, corresponding to a concentration of ~19 mM), it is surprising (and, for the moment, not fully understood) that the Dex-Phos flux did not decrease somewhat after 6 hours of current passage. From a practical standpoint, however, this is a positive result, as an iontophoretic delivery device for Dex-Phos would not require complex characteristics, such as an ion-exchange membrane to sequester Cl⁻.

Other Practical Issues

The steady-state flux of Dex-Phos from water (~70 nmol/h at a current of 0.3 mA, or ~2 μg/mA-min) translates to the delivery of about 0.16 mg from a typical current “dose” of 80 mA-min. Although this is rather less than that administered via injection (1 mL of a 0.4% weight-to-volume ratio solution contains 4 mg), it is significantly greater than that resulting from iontophoresis of the Dex-Phos formulations containing citrate (~0.02 mg) and lidocaine (again, ~0.02 mg), as reported previously.

In vitro, the current “dose” typically is delivered over 20 minutes, and a steady-state flux is unlikely to be achieved in this time span (in the experiments reported here [data not shown], a lag time of about 30–60 minutes was observed). The kinetics of transport and uptake in vitro are typically more rapid than those seen in vivo due to the presence of a fully functional microcirculation, which can “clear” the drug to the underlying tissue and, ultimately, the systemic circulation. Furthermore, it is known that in vivo uptake and absorption can be influenced significantly by, for example, local vasoconstriction.

Clinical Implications

The simple in vitro model used in this study cannot perfectly replicate the much more complex in vivo situation in humans. However, porcine skin, even when stored frozen before use, is a good model for human skin. The trends observed here also may be expected in vivo. To optimize Dex-Phos delivery, the drug must be delivered from the cathode, and the formulation should exclude, as far as possible, the presence of competing co-ions. If commercially available Dex-Phos solutions are used, preference should be given to those that contain the least amounts of anions such as chloride and citrate. Ultimately, well-designed in vivo studies will be necessary to demonstrate the efficiency of iontophoresis to deliver clinically relevant corticosteroid doses in soft tissues, and the results presented here should be of particular importance for their design.
In Vitro Optimization of Dexamethasone Phosphate Delivery by Iontophoresis

Frank B Underwood

Iontophoresis of corticosteroids in the management of local inflammatory conditions is a controversial topic. The available evidence regarding the clinical efficacy of iontophoresis is contradictory and generally of low quality, with few notable exceptions. The research by Sylvestre and colleagues1 provides much-needed clarity to the technical aspects of this treatment and should serve as a stimulus to and basis for high-quality clinical trials.

In 1992, Petelenz et al2 determined in laboratory studies that dexamethasone is most effectively delivered via electromigration from the cathode of a direct current circuit. Despite their findings, many reference textbooks for electrotherapy used in professional (entry-level) physical therapist education programs persisted in listing dexamethasone as a positively charged ion. It is encouraging that most current textbooks (eg, Cameron,3 Prentice,4 Michlovitz and Nolan,5 Hayes and Nelson6) now clearly state that dexamethasone has a negative charge in solution and, therefore, should be delivered from the cathode. Despite this readily available information, some re-
In Vitro Optimization of Dexamethasone Phosphate Delivery by Iontophoresis
Jean-Philippe Sylvestre, Richard H Guy and M Begoña Delgado-Charro
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