Quantification of Total Dexamethasone Phosphate Delivery by Iontophoresis

Abstract

The total amount of dexamethasone phosphate transferred into the human body as a function of iontophoresis has not previously been determined, despite its widespread clinical use in the treatment of localized inflammation. The objective of this study was to document the optimal parameters required for clinical iontophoresis of dexamethasone phosphate.

Results were achieved by the experiment of in vitro evaluations of dexamethasone phosphate iontophoresis and by in vivo estimations of drug amounts (milligrams) iontophoresed into healthy human volunteers. The in vitro evaluations were conducted to quantify total dexamethasone phosphate amounts transferred as a function of dosage (milliAmp-minutes), to evaluate the efficiency of the delivery based on dexamethasone phosphate only (pure) donor solutions compared with dexamethasone phosphate+ salts (coformulated) donor solutions, and to compare the delivery from the negative electrode (cathode) with that from the positive electrode (anode). The in vivo drug amounts were estimated by the use of the formulation conditions determined from the in vivo testing. The in vitro evaluations were conducted with side-by-side glass diffusion cells, which measured iontophoretic and passive delivery across an ultrafiltration membrane. The in vivo were conducted on five healthy human volunteers who were wearing a low-voltage iontophoresis system. Total drug delivery was ascertained by the difference between the initial drug load and a final residual amount determined by extraction.

The in vitro results demonstrated increased dexamethasone phosphate delivery with higher iontophoretic dosages and with the pure dexamethasone phosphate formulation. Delivery from the anode was significantly lower than that from the cathode. After an 80-mA-minute drug-delivery dosage was administered, the in vivo iontophoretic delivery was measured to be 1.40 ± 0.23 mg, and the corresponding passive delivery was 0.26 ± 0.16 mg.

The in vitro experiments confirm iontophoretic delivery of dexamethasone phosphate across artificial membranes, and the in vivo experiments suggest that drug is delivered into human skin.

Introduction

The use of bipolar electric fields (iontophoresis) as a means to propel ionic drug molecules noninvasively across the skin and into underlying tissue is a commonly practiced modality in physical therapy. A significant number of these applications utilize iontophoresis of negatively charged dexamethasone phosphate (DEX-P), the phosphorylated prodrug of dexamethasone (DEX), in the localized treatment of inflammation. There are many reports in the literature examining the clinical efficacy of this mode of drug delivery and its effectiveness in treating inflammation in different areas of the body. Despite the widespread clinical use of DEX-P iontophoresis, there is little published scientific literature on DEX-P pharmacokinetics following iontophoresis. The justification for DEX-P iontophoresis is generally provided by two widely cited animal, ie, monkey and pig, studies. Unfortunately, these iontophoretic studies used methodology or measured endpoints that are not relevant to current clinical iontophoresis. These studies were also widely disparate in measured DEX/DEX-P delivery amounts. Glass et al reported that delivery averaged 10 µg DEX/DEX-P after a 100 mA-minute iontophoretic dosage. In contrast, Petelenz et al reported that approximately 30 µg of DEX/DEX-P was delivered from an 80-mA-minute dosage. Several methodological differences existed between these studies. Glass et al mixed DEX-P with competing ions and used the anode. Both of these practices are now discouraged in general practice since DEX-P is negatively charged. Confusion still exists as to the appropriate polarity of the electrode for delivery since DEX-P, stated as dexamethasone sodium phosphate, may be misinterpreted as a cationic phosphate salt rather than an anionic sodium salt.

Smutok et al investigated DEX-P delivery by iontophoresis into the wrist of human volunteers. Iontophoresed drug was determined by high-performance liquid chromatographic (HPLC) analysis of plasma extracted from the proximal
effluent venous blood. These investigators were unable to detect either DEX-P or DEX during iontophoresis and up to 120 minutes following iontophoresis completion. Their results suggest that amounts of DEX and/or DEX-P delivered to the bloodstream may be below the detection limits of HPLC.

The present study was designed to quantify how much DEX-P is actually transferred to the body by iontophoresis and to optimize parameters required for clinical DEX-P iontophoresis. Results were achieved by the experiments of in vitro DEX-P delivery evaluations and by in vivo estimations of drug amounts iontophoresed into healthy human volunteers. The in vitro DEX-P delivery evaluations were conducted to quantify the total DEX-P transferred amounts as a function of iontophoretic dosage applied, to evaluate the efficiency of the delivery based on the pure DEX-P compared with the coformulated DEX-P donor solutions, and to compare the delivery from the cathode with the delivery from the anode. The in vivo drug amounts were estimated by the use of the optimal conditions determined from the in vitro testing.

Materials and Methods

In Vitro Delivery Studies

The in vitro iontophoresis test apparatus (Figure 1) is a glass side-by-side diffusion cell (Crown Glass, Inc, Somerville, New Jersey). The donor cell was filled with 9 mL of 4 mg/mL of pure or coformulated DEX-P (Sigma Chemical Co, St. Louis, Missouri), and the receiver cell was filled with 9 mL of 1% NaCl. To restrict passive drug flow and mimic a stratum corneum barrier, a synthetic membrane (Millipore Corp, Bedford, Massachusetts) separated donor and receiver chambers. A previous study\textsuperscript{14} has shown that synthetic membranes can be used in vitro to evaluate iontophoretic delivery of corticosteroids. The ultrafiltration membrane used in this experiment had a nominal molecular weight limit of 1000, which would retain molecules greater than a molecular weight of 1000 while allowing smaller molecules to pass through. A 1-V iontophoresis power source (Birch Point Medical, Inc, St. Paul, Minnesota), consisting of a silver/silver chloride (Ag/AgCl) cathode and zinc (Zn) anode, was positioned in the cell (Figure 1). To monitor current flow as a function of time, cumulative dosage (milliAmp-minute), a digital multimeter (RadioShack 22-168A, Fort Worth, Texas) was connected in series with the electrodes. Passive drug delivery was monitored using a duplicate side-by-side diffusion cell, which was run without the power source. Samples of the passive drug delivery and the active system were taken at the same time points. A total of four side-by-side evaluations was conducted in each experiment. To evaluate iontophoretic delivery as a function of dosage applied, the amount transferred into the receiver chamber from a cathode was measured after 40-, 80-, and 120-mA-minute dosages were administered. To evaluate iontophoretic delivery as a function of DEX-P solution purity, an 80 mA-minute delivery from a 4-mg/mL pure DEX-P solution was compared to that from a 4-mg/mL DEX-P solution coformulated with 10 mg/mL sodium citrate as a buffer. To evaluate iontophoretic delivery as a function of donor electrode polarity, the delivery of DEX-P utilizing an 80-mA-minute dosage from a cathode was compared with that from an anode under the same experimental conditions.

In Vivo Experimental Design

By means of a repeated measures design, each subject was exposed to active DEX-P iontophoresis from the cathode and passive delivery for an equivalent period of time. A minimum of 1 week separated the wearing of active and passive electrode sets.

Human Subjects and In Vivo Iontophoresis

The Institutional Review Board of East Tennessee State University and/or Veteran’s Administration approved this study, and each subject gave written informed consent. The rights of the subjects were protected. Experiments examining the transfer of DEX-P as a result of iontophoresis were conducted on five Caucasian human volunteers, ie, four men and one woman ranging in age from 34 to 43 years.

The iontophoresis system used was the IontoPatch (Birch Point Medical, Inc), which has a self-contained power source of 80 mA-minute capacity. Total drug transferred to the subject during the iontophoresis was estimated by measuring the difference between a known starting amount of DEX-P, as loaded into a delivery electrode, and its final residual amount following iontophoresis. Each delivery electrode was loaded

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**Figure 1. In Vitro Iontophoresis Apparatus.**

Donor Cell

Negative Electrode

Ultrafiltration Membrane

Receiver Cell

Positive Electrode

Note: When the cathode was used to deliver dexamethasone phosphate, the donor compartment contained a silver/silver chloride electrode, and the receiver compartment contained the zinc anode. The positions in the donor and receiver compartments were reversed for the electrodes when the anode was used to deliver dexamethasone phosphate.
with a known amount of drug as determined by the gravimetric weight of an amount between 5.04 to 5.77 mg, in the form of a 4.0-mg/mL DEX-P solution. The return electrode was filled with a 0.9% saline solution. The electrode set was then placed over the volunteer’s lower leg, ie, just above the ankle, with the delivery electrode placed lateral to the midline. To control possible interference by any chemical species generated as a result of direct current or species absorbed by the electrodes from the skin, “blank” (saline-filled) electrode sets were used simultaneously. The blank iontophoresis was conducted on the contralateral leg location using an identical electrode set, but the delivery electrode was loaded with 0.9% sodium chloride solution. The iontophoresis systems were worn for 24 hours in accordance with the manufacturer’s recommendations. Passive delivery was evaluated by use of identical electrode sets, with the power sources electrically disconnected in both drug-containing and blank electrodes. The application period was the same as for the iontophoretic delivery. Following all procedures, the active and blank iontophoresis and the passive and blank applications electrode sets were removed from the volunteer, and skin sites under the delivery electrode locations were wiped with a cotton pad to remove any superficial drug remaining on the skin surface. The delivery electrodes and cotton pads were then extracted and analyzed for residual drug content by absorbance difference between the active and blank extraction solutions.

Drug Extraction and Analysis

In vitro iontophoretic delivery was determined by spectrophotometric (Cecil Instruments, Model CE 2041, Cambridge, England) analysis of receiver-cell aliquots and comparison of absorbance at 239 nm to a known drug standard curve. Following in vivo iontophoresis, DEX-P delivery was determined following extraction of the drug remaining in the iontophoretic patch and cotton pad. Extraction was accomplished by immersing the drug-containing cathode in a container of 500 mL of distilled water and periodically stirring the mixture over the course of a 24-hour period. The extraction and analysis method was evaluated for validity by loading patches with known amounts of either DEX-P (n=6) or DEX (n=6) and determining percent recovery by analysis of the extracted solution. As with in vitro studies, DEX/DEX-P content in the extract solution was determined by spectrophotometric analysis.

Statistical Analysis

All data are presented as mean ± standard error of the mean. Appropriate inferential statistical analyses were performed. The a priori level of significance between two groups was established as $P < 0.05$.

Results

In Vitro Experiments

The spectrophotometric analysis was equally sensitive to both DEX and DEX-P. Standards of both forms showed
equivalent molar absorptivities at the wavelength used. The percentage of recovery for the extraction and analysis procedure was measured to be 99.2 ± 0.2% for DEX-P and 100.2 ± 0.9% for DEX. Cathodic delivery of DEX-P as a function of iontophoretic dosage applied is illustrated in Figure 2. Delivery in the active iontophoresis cell was 0.51 ± 0.05 mg at 40 mA-minute, 0.78 ± 0.11 mg at 80 mA-minute, and 0.94 ± 0.15 mg at 120 mA-minute. At the end of an equivalent time period similar to 120 mA-minute, the passive delivery was 0.04 ± 0.01 mg. Delivery of an 80 mA-minute dosage of DEX-P from the active electrode as a function of solution purity is illustrated graphically in Figure 3. Cathodic delivery from the citrate coformulated version was 0.18 ± 0.04 mg compared with 0.78 ± 0.11 mg from the pure DEX-P formulation. An approximate fourfold greater delivery occurred when the pure DEX-P was used, compared with the coformulated version (P < 0.011, n=12 per group). Under otherwise identical conditions, anodic iontophoresis with the pure and coformulated DEX-P was 0.015 ± 0.002 and 0.019 ± 0.006 mg, respectively. A significant increase in delivery occurred from the cathode, compared with the anode (P < 0.001 and P = 0.002 for the pure and coformulated versions of DEX-P, respectively, using a t-test with n=4 per group). Anodic delivery was not significantly different from an equal time period of corresponding passive delivery.

In Vivo Experiments

The results of the in vivo investigation with human volunteers are shown in Figure 4. During active cathodic iontophoresis, 1.40 ± 0.23 mg of DEX-P was lost from the delivery electrode. The amount transferred during a similar passive period was 0.26 ± 0.16 mg.

Discussion

The in vitro experiments demonstrated greater amounts of DEX-P delivery as the iontophoretic dosage (milliAmp-minutes) was increased. Unexpectedly, we found a nonlinear relationship, which indicates diminishing delivery efficiency as dosage increases. We hypothesize that extraneous ions are introduced into the donor chamber as current flows in this experiment, which reduces efficiency as the new anions compete with DEX-P for delivery into the receiver chamber. These anions may have been introduced by the cathodic delivery electrode—in this case Cl− anion released in the AgCl reduction reaction at the electrode surface.

The in vitro results also demonstrate the importance of donor solution composition. We compared the delivery efficiency of a pure DEX-P donor solution with the delivery of a coformulated DEX-P donor solution. A significant decrease in delivery was accomplished when DEX-P was coformulated. The decreased delivery is presumed to be due to current-carrying competition between anions of citrate and DEX-P. A pure DEX-P formulation is commercially available from compounding pharmacies. A coformulated DEX-P was made similar to Decadron-phosphate.

By the use of Faraday’s constant and the total charge transferred in this experiment, it was estimated that the percentage of the iontophoretic current attributable to drug transfer was 6.7% and 9.7% for our in vitro and in vivo experiments, respectively. While these percentages are low, they are generally within the range expected for iontophoresis efficiency for drugs of this molecular weight. The majority of current with iontophoresis is attributable to factors other than drug transfer. Two examples of these attributing factors are as follows:

- Movement of competing ions into the body (ions of like charge to the drug molecule) emanating from sources such as the electrode surface
- Movement of counter ions of opposite charge (such as sodium ion) transferred from the body to the delivery device

We hypothesize that since we used a common donor electrode, the decreased efficiency noted in our in vitro experiment was due to a greater degree of sodium ion “back transfer” than was present in the in vivo experiment. Further testing would be required to validate this hypothesis. The in vitro experiments and the in vivo experiments document significantly greater iontophoretic delivery compared to passive delivery. The amounts of DEX-P iontophoresed in vivo (average = 1.4 mg) are theoretically within the range suggested for localized treatment of inflammation by intra-articular injections (0.8 to 4 mg), intralesional injections (0.2 to 6 mg), or soft-tissue injections (0.4 to 6 mg).

Although the results of our in vivo experiments suggest drug delivery across the skin, we are cognizant of the fact that the drug was not actually measured in the body. However, the careful recovery experiments support the logical conclusion that the “lost” drug was transferred into the skin. Further
support for this interpretation comes from a previous investigation of transdermal delivery of fentanyl, where an average of 92% of the fentanyl lost from a passive-delivery patch was eventually “found” absorbed into the systemic circulation.18

Lillich et al16 hypothesized that DEX-P concentrations in the range of 30 ng/mL may be sufficient to have a modulatory effect on inflammatory mediators in the synovium. Our current investigation indicates the potential for clinically effective DEX-P amounts to be delivered across the skin and into the body with a low-energy iontophoretic system. However, further studies are needed to elucidate the pharmacokinetics of the drug as it penetrates into the subcutaneous tissue following iontophoresis.

Conclusion

The following conclusions appear warranted from this study. These experiments document iontophoretic delivery of DEX-P across artificial membrane, as well as into human skin. Higher amounts of DEX-P are delivered with higher iontophoretic dosages. Evidence is also presented that suggests this relationship may be nonlinear. Pure formulations of DEX-P are substantially more efficient than coformulations that contain competing anions. Concentrations of DEX-P are significantly greater when delivered from the cathode than when delivered from the anode. In vivo DEX-P iontophoresis may deliver drug quantities approximating those used in syringe injection. However, further investigation will be required to delineate the DEX-P iontophoretic pharmacokinetics.

References


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