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Effects of Ice Massage Prior to an Iontophoresis Treatment Using Dexamethasone

Brady Michael Smith

A thesis submitted to the faculty of Brigham Young University in partial fulfillment of the requirements for the degree of

Master of Science

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ABSTRACT

Effects of Ice Massage Prior to an Iontophoresis Treatment Using Dexamethasone

Brady Michael Smith Department of Exercise Sciences, BYU Master of Science

Context: Low current intensity iontophoresis treatments have shown an increase in skin perfusion over 700% from baseline potentially increasing drug clearance from the targeted area.

Objective: To determine the effects of a 10-minute ice massage on subcutaneous dexamethasone sodium phosphate (Dex-P) concentration and skin perfusion during and after a 4 mA iontophoresis treatment.

Design: Controlled laboratory study.

Setting: Research Laboratory.

Patients or Other Participants: 26 individual participants (Males = 15, Females = 11, age = 25.6 ± 4.5 y, height = 173.9 ± 8.51 cm, mass = 76.11 ± 16.84 kg).

Interventions: Participants were randomly assigned into two groups: 1) Pretreatment 10minute ice massage; and 2) no pretreatment ice massage. Treatment consisted of an 80 mA·min (4 mA·20 minutes) Dex-P iontophoresis treatment. Microdialysis probes (3 mm deep in the forearm) were used to assess Dex-P, dexamethasone (Dex), and its metabolite (Dex-met) concentrations. Skin perfusion was measured using laser Doppler flowmetry probes.

Main Outcome Measures: Microdialysis samples were collected at baseline, at conclusion of treatment, and every 20 minutes posttreatment for 60 minutes. Samples were analyzed to determine Dex-total concentration ([Dex-total] = Dex-P + Dex + Dex-met). Skin perfusion was calculated as a percent change from baseline. A repeated measures ANOVA was used for Dex-total and Skin Perfusion.

Results: No significant difference was found in [Dex-total] between ice and no ice treatments, (P = 0.265). A significant increase in [Dex-total] occurred over the course of the iontophoresis and posttreatment time (P < 0.0004). Dex-P was recovered in 15 of 21 participants with a mean concentration of $0.604 \pm 0.843 \mu g/mL$. Peak skin perfusion reached $27.74 \pm 47.49\%$ and $117.39 \pm 103.45\%$ from baseline for the ice and nonice groups, respectively.

Conclusions: The 10-minute ice massage prior to iontophoresis does not significantly alter the delivery of [Dex-total] through the skin. A greater [Dex-P] was recovered than previously seen with lower intensities.

Keywords: iontophoresis, transdermal drug delivery, skin perfusion, dexamethasone, microdialysis

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INTRODUCTION

Iontophoresis is a *noninvasive* technique that enhances ionized drug penetration through the skin using a small electrical current. The current provides an ion-electric flow, creating a force that drives the ionized drug through the skin, increases the skin's permeability, and promotes electro-osmosis.^{3,29} Currently, few studies have directly measured iontophoresis delivery in vivo in human studies. Further research is needed to determine the most efficient treatment techniques to maximize iontophoresis drug delivery.

Dexamethasone sodium phosphate concentration delivered via iontophoresis has been examined through different measurement and distribution techniques. Direct tissue concentrations have been assessed using tissue biopsies¹³ and microdialysis.³⁰ Skin blanching has been used to indirectly assess Dex-P delivery due to the drugs vasoconstriction properties.² Following a 40 mA min iontophoresis treatment at a 4 mA intensity for 20 minutes, Gurney et al¹⁴ recovered Dex-P in 43% of all biopsy samples, but Rigby et al³⁰ recovered no Dex-P using a 120 mA min treatment at intensities of 1 or 2 mA. Anderson et al,² observed cutaneous vasoconstriction skin blanching on all participants when using a 0.1 and 4 mA current intensity.

The electro-current produced by an iontophoresis treatment increases skin microcirculation.^{4,8,30,32} Cathodal iontophoresis causes up to a 700% (from baseline) increase of skin perfusion produced by current-induced vasodilation.^{4,30} It is hypothesized that during this large increase in microcirculation drug washout occurs (clearing the drug from the target tissue), decreasing the drug's effectiveness.³⁰ The possibility exists that introducing an intervention that decreases vasodilation, may lower drug washout from the target tissues.

Cryotherapy produces many different physiological effects including the ability to decrease blood perfusion.^{1,10,16,17,19,21,22,24,33} Ice application and cold water immersion have

reduced both arterial blood flow and metabolism.^{16,17,22-24} Applying an ice wrap (0°C to 1°C) for 20 minutes to the skin of the knee decreased soft tissue blood flow by an average of 25.8% and arterial blood flow by an average of 38%.¹⁷ Skin perfusion was decreased 76% at a depth of 2 mm after a 7-minute cryo/cuff treatment.²⁴

The purpose of this study was to determine if cryotherapy, when used prior to an iontophoresis treatment, would influence tissue concentration of Dex-P, its biologically active form dexamethasone (Dex), and its metabolites (Dex-met). We hypothesized that a 10-minute ice massage prior to an iontophoretic treatment would lead to a greater concentration of Dex-total at the target tissue.

METHODS

We used a randomized controlled laboratory design for this study. The independent variables included two treatment groups (10-min pretreatment ice massage; controlled treatment without 10-min ice massage) and time intervals (20-min baseline, 20-min treatment, 60-min post treatment in 20-min intervals). The dependent variables measured in this study were skin perfusion and total dexamethasone concentration ([Dex-total]).

Participants

Twenty-six healthy individuals (Males = 15, Females = 11, age = 25.6 ± 4.5 y, height = 173.9 ± 8.51 cm, mass = 76.11 ± 16.84 kg) were recruited and enrolled into this study. We excluded those who were pregnant from this study due to potential side effects of Dex-P on fetal development. Female subjects were required to take a pregnancy test in order to participate. Other exclusion criteria included: Subjects who had a known allergy to Dex, were diabetic, had decreased circulation in forearm and hand, had an infection or open wound on the forearm, any skin, liver, kidney, or pancreatic disorders or an injury to either arm within the past two months.²

The study was reviewed by the Brigham Young University Institutional Review Board before participants were recruited and enrolled in the study. All participants were provided written informed consent, which they signed, before taking part in the study.

Instrumentation and Dialysate Analysis

Dexamethasone sodium phosphate was delivered to the treatment site using the Trivarion iontophoresis delivery kit (North Coast Medical, Inc., Gilroy, CA). The cathodal electrode was prepared with 2 ml of 0.4% Dex-P and placed 15 cm (6 in)^{23,30} distal to the center of the dispersive electrode. An ActivaDose II dose controller (North Coast Medical, Inc., Gilroy, CA) was used to deliver the iontophoresis treatment.

Microdialysis probes were manufactured in our laboratory using 13 Kilo-Dalton linear (3.0 cm) hollow fiber and were gas sterilized using ethylene oxide. The microdialysis probes were perfused with sterile saline using an infusion pump (model: Pump 11 VPF; Harvard Apparatus, Holliston, MA). The depth of each microdialysis probe inserted into the treatment site was measured using musculoskeletal ultrasound imaging (model: LogiQ 5e, General Electric Company, Fairfield, CT). On average, microdialysis probes were placed under the skin at a depth of 3.1 ± 0.94 mm.

We used reverse-phase, high performance liquid chromatography (RP-HPLC) to measure the concentrations of Dex-P, Dex and its metabolite (Dex-21-oic acid) using a previously established method.³⁰ A diode array detector (model: 1260 Infinity, Agilent Technologies, Inc., Santa Clara, CA), using a wavelength of 239 nm, was used to measure the peaks of Dex-P at 4.2 minutes, Dex at 12.4 minutes, and Dex-21-oic acid at 5.8 minutes. We quantified the lower limits of Dex-P and Dex being 100 ng/mL and 50 ng/mL, respectively. Skin blood flow was measured using laser Doppler flowmetry (LDF). Two laser Doppler probes (model: VP7a, Moore Instruments, Wilmington, DE) were interfaced with a PowerLab data acquisition system (ADInstruments Inc., Colorado Springs, CO) to measure relative changes in the skin blood flow. Our LDF parameters were a time const-0.5 s and 5.0 V = 1000PU.

Reusable cryocups were used to cool the treatment area prior to drug delivery. Each cryocup was previously filled (with tap water) and frozen at approximately -18° C (0°F). The cryocup included a cold-retardant handle and contoured base for easier application (Figure 1). *Procedures*

Participants reported for a single treatment to the Therapeutic Modalities Lab at our university. Each individual was screened for the inclusion and exclusion criteria. Participants were randomly assigned into one of the two treatment groups: 1) 20-minute iontophoresis treatment with a 4 mA current intensity without a 10-minute ice massage or 2) 20-minute iontophoresis treatment with a 4 mA current intensity with a 10-minute ice massage prior to treatment. Group assignments were selected through a random draw.

Similar procedures were performed and described by Rigby et al.³⁰ Two microdialysis probes were inserted below the surface of the skin at a target depth of 3 mm, due to the approximate superficial depth of commonly treated tendons that use Dex-P iontophoresis when diagnosed as a tendinopathy.⁵ Though probe placement into the tendon would have the potential to yield more clinically relevant results, extensive damage could be caused in the tendon. Therefore, the forearm was used in this study.

Each subject was seated in a recumbent chair. The area of the posterior forearm with the largest visual girth was chosen as the treatment site. A 25.5 x 13.25 cm area was trimmed with hair scissors and wiped with an alcohol prep pad. With a felt marker, we marked the forearm

indicating where the cannula needles were to be inserted and exited from the skin (5 cm distance between insertion and exit sites). The treatment area was cleansed with an iodine swab.

Two sterile 9 cm 27-gauge pediatric spinal tap needles (reference #40581, BD Company, Franklin Lakes, NJ) were inserted into the subcutaneous tissue of the subject's forearm. Using musculoskeletal ultrasound imaging, we verified the depth of the needles. With the needles in place, we inserted the microdialysis probes through the needle and then the needles were removed.

Pretreatment dialysate was collected by perfusing sterile saline solution through the probe at 1.3 μ L/min for 60 minutes. This pretreatment period allowed for the tissue to recover from the trauma of having the needles and probes inserted at the target depth. The last 20 minutes of the pretreatment period was used to measure baseline drug concentration.

After the 60-minute recovery period, the participants in the cryotherapy treatment group were treated with a 10-minute ice massage using a cryocup. A 10-minute treatment was chosen based on findings of multiple studies.^{17,34} We applied the ice massage with back and forth stroke movements and medium pressure over the treatment area. We applied medium pressure by pressing the cryocup just enough for the skin to begin to be indented. After the ice massage treatment, the treatment area was patted dry with a towel and then cleansed with an alcohol prep pad prior to applying the iontophoresis electrodes and LDF probes.

The cathode electrode was prepared with 2 mL of 0.4% Dex-P and placed directly over the microdialysis probes. The dispersive electrode was placed distally 15 cm on the forearm. The LDF probes were placed within the cathodal electrode. One LDF probe was placed within the center of the drug reservoir and the other on the periphery outside of the drug reservoir (approximately 2 cm apart). The electrode (with LDF probes) was placed over the microdialysis

probes in a way that allowed the microdialysis probes to run between the LDF probes (Figure 2). Immediately after placing the LDF probes on the skin, a 1-minute blood flow baseline measurement was recorded.

With the leads attached to their respective electrodes, the dose controller was turned on to 4 mA for 20 minutes (80 mA·min). At the end of the 80 mA·min treatment, the iontophoresis device was shut off and the electrodes were left on the skin for the remainder of the posttreatment collection period. With the perfusion rate at 1.3 μ L/min, dialysate was collected from the microdialysis probes in 20-minute intervals for the 20-minute treatment and 60 minutes following the treatment (collection took place at minute 20, 40, and 60 posttreatment). The samples were stored immediately after collection in a -20° C freezer until analyzed using RP-HPLC. We recorded skin perfusion continuously throughout the 20-minute iontophoresis treatment and 60-minute posttreatment.

DATA ANALYSIS

RP-HPLC Analysis

Standard curves for Dex-P and Dex were examined at the beginning of the data collection process. We did not have a standard for Dex-Met (Dex-21-oic acid), therefore, the mean curve between Dex-P and Dex was used for the analysis of Dex-met).

Statistical Analysis

Dexamethasone Tissue Concentration. Using the RP-HPLC standard curve and retrodialysis recovery values derived from Rigby et al,³⁰ we calculated the in vivo Dex-total (Dex-total = Dex-P + Dex + Dex-met) concentrations for each subject at each time point. We used a repeated ANOVA to analyze differences in Dex-total between the two treatment conditions during the 20-minute iontophoresis treatment and 60-minute posttreatment.

Skin Perfusion. Skin perfusion data was averaged every 20 minutes. Changes in skin perfusion were expressed as a percent change in skin perfusion relative to baseline. We used a repeated measures ANOVA to analyze differences in normalized skin perfusion between the two treatment conditions during the iontophoresis treatment and posttreatment periods.

Tukey-Kramer post hoc testing procedures were used for both analyses of variance. We used JMP Statistical Software (JMP Pro 10 ISAS Inc., Cary, NC), for all statistical analyses, and the alpha level was set at P < 0.05.

RESULTS

The accumulation of Dex-total as a function of iontophoresis dose is shown in Figure 3 for the different treatment groups. Data from subject 13 (ice group) and 14 (no ice group) were excluded, as the data was thought to have been tainted with external Dex-P entering the microdialysis probes near the portal sites. Data from subjects 21 to 23 (ice group) was not analyzed correctly in the HPLC lab. Thus, there was an unequal N for the treatment groups when analyzing Dex concentration data (ice = 9, nonice = 12).

As seen in TABLE 1, we recovered Dex-P in 15 of 21 subjects during both treatment and posttreatment times with a mean concentration of $0.604 \pm 0.843 \ \mu\text{g/mL}$. Dex was found in 6 of 21 subjects during both treatment and posttreatment times with a mean concentration of $1.256 \pm 1.590 \ \mu\text{g/mL}$. Finally, 14 of 21 subjects were found to have measurable levels of Dex-met during both treatment and posttreatment times with a mean concentration of $2.673 \pm 1.940 \ \mu\text{g/mL}$. The total mean concentration of Dex-P (2.559 \ \mu\text{g/mL}), Dex (0.967 \ \mu\text{g/mL}), and Dex-met (10.779 \ \mu\text{g/mL}) of all subjects collected, represent 17.89%, 6.77%, and 75.34% of [Dex-total] (14.306 \ \mu\text{g/mL}), respectively.

On average, there was no difference in tissue [Dex-total] between ice and no ice treatments (treatment main effect) ($F_{1,19} = 1.32$, P = 0.265). Across the treatment and posttreatment time, there was no difference in tissue [Dex-total] between ice and no ice (treatment x time interaction) ($F_{4,76} = 0.51$, P = 0.725). There was a significant increase in tissue [Dex-total] over the course of the iontophoresis and posttreatment time (time main effect) ($F_{4,76} =$ 5.7313, P = 0.0004). Tissue [Dex-total] at the conclusion of the 80 mA·min iontophoresis treatment for the ice and nonice groups was 1.02 ± 2.07 and $0.78 \pm 0.83 \mu g/mL$, respectively. [Dex-total] for both treatments increased throughout the treatment and posttreatment times. Mean concentrations for the ice and nonice treatment groups were 2.25 ± 2.09 and 3.76 ± 4.88 , respectively. Significantly greater concentrations of Dex-total occurred at 60 minutes posttreatment intervals. Significantly greater tissue [Dex-total] occurred at 40 minutes posttreatment intervals. Significantly greater tissue [Dex-total] occurred at 40 minutes

On average, skin perfusion was different between the two treatment groups (treatment main effect) ($F_{1,24} = 7.24$, P = 0.0128). Skin perfusion had a greater increase in the nonice treatment group during and immediately after the iontophoresis treatment (treatment x time interaction) ($F_{4,96} = 22.73$, P = 0.0001) (Figure 5). Peak skin perfusion of both the ice (27.74 ± 47.49% of baseline) and nonice (117.39 ± 103.45% of baseline) treatment groups occurred at the end of the 20-minute iontophoresis treatment. Skin perfusion returned to baseline values 20 minutes after the iontophoresis treatment of the ice group, whereas, the nonice group returned to baseline values 60 minutes posttreatment.

DISCUSSION

Iontophoresis enhances the permeability of the skin allowing ionized drugs to pass through it more freely. This enhanced permeation is caused by electromigration, electro-osmosis, and passive diffusion.¹⁸ Electromigration is a result of like ions repelling one another, creating an ion-electric flow that drives ionized drugs through the skin, increases the permeability of the skin, and promotes electro-osmosis.^{23,25} Once the stratum corneum, a lipid bilayer of skin, has reached a specific threshold (approximately 60 V) this layer of skin becomes less resistant and more permeable to smaller ionic molecules (eg, Na⁺, Cl⁻, Ca²⁺). Although the stratum corneum acts as a restrictive barrier to exogenous molecules, its many pores can act as conduction pathways when an electrical current is applied.²¹

We hypothesized that cryotherapy would lower skin perfusion due to its vasoconstriction properties preventing drug clearance from the target tissue. However, we found no difference in [Dex-total] when comparing a pretreatment ice massage group with a nonice massage group. Although there was no difference in [Dex-total] between the two groups, there was an increase in skin perfusion throughout the treatment period for the nonice massage group over the ice massage group. Cathodal iontophoresis, using low intensities (≤ 2 mA), has been shown to enhance the transdermal delivery of Dex-P,²⁷ while increasing skin perfusion by more than 700%.^{4,30} Our study saw a significant increase in skin perfusion, but only reaching a peak value of 117.39% in the nonice group. Lower skin perfusion peak values may be explained by the shorter treatment time used for the same iontophoresis dose when a higher current is used. We hypothesized that increased skin perfusion has the ability to aid drug delivery as long as it does not reach a certain threshold, causing drug clearance from the intended tissue. This theory is supported when comparing our study that collected higher [Dex-P] and [Dex] to a similar study that reported skin perfusion levels to be more than 700% from baseline.³⁰

An advantage of iontophoresis is that varying treatment intensity and dosage can control drug delivery kinetics.^{20,28} The ionic flow created by the chosen current and duration had the ability to produce more repulsion or less repulsion of a drug. We used an 80 mA·min treatment dose with an intensity of 4 mA leading to a 20-minute treatment. With this dosage and intensity, the majority of [Dex-total] recovered was Dex-met (75.34%), the metabolite of Dex known as Dex-21-oic acid.⁶ Dex-P concentrations were recovered throughout the treatment and/or posttreatment times in 15 out of 21 participants. Using the same microdialysis collection technique, Rigby et al³⁰ also found the largest Dex-total component to be Dex-met, but discovered no accumulation of Dex-P under the skin when using a 120 mA·min (1 and 2 mA intensities) iontophoresis treatment. Other previous studies,^{2,13,14} using a dosage of 40 mA·min, with intensities between 0.05 and 4 mA, have shown varied results in the amount of [Dex] and/or [Dex-P] recovered. Gurney et al¹³ found 7 of 16 subjects with an average [Dex-P] of 2.9 ng/g. The higher intensity used in our study may be more effective in creating an electromigration pathway for the ionized Dex-P.

Dex-P remains negatively charged until it enters the skin and is dephosphorylated into its active form, Dex.^{27,30} When Dex-P does not fully hydrolyze into its pure Dex form, it transforms into an acid ester form known as Dex-21-oic acid.⁶ Previous studies^{13,14,30} using various dosages (120 mA·min and 40 mA·min) have found low concentrations of Dex in the targeted tissue area. For example, Rigby et al³⁰ recovered [Dex] in 6 of 32 participants with a mean concentration of 109.9 ± 88.8 ng/mL. Similarly, we found [Dex] in 6 of 21 subjects with a mean concentration of 967.0 ± 0.448 ng/mL, representing just 6.77% of Dex-total. Heiss et al¹⁵ established the median

effective dose (ED_{50}) of Dex to be 75 ng/g, while lower concentrations of Dex can still manifest an anti-inflammatory effect. The anti-inflammatory properties of Dex are efficient at clinically relevant delivery concentrations to superficial tendons as the [Dex] exceeds the ED_{50} by the end of the 60-minute posttreatment.

Although, the primary iontophoretic transportation of a drug occurs through the shunt pathway and paracellular routes¹⁸ (eg, sweat glands and hair follicles), deeper infiltration occurs due to passive diffusion and transportation via the microvascular system.^{2,7,11,30} This can be observed as the [Dex-total] continues to increase posttreatment in both the ice and nonice groups (at an average depth of 3.1 ± 0.94 mm). In a similar study³⁰ measuring [Dex-total] at 1 and 4 mm, [Dex-total] began to decrease shortly after the iontophoresis treatment ended. Two notable differences can be distinguished between these two studies. The first, a higher current, shorter duration treatment was used in our study compared to the low current, long duration treatment of Rigby et al.³⁰ It has been suggested that local blood flow determines systemic and underlying tissue solute absorption but not epidermal penetration fluxes during iontophoretic delivery.⁷ As skin perfusion had begun returning to baseline shortly after the treatment, microcirculation seemed to be the likely cause for passive diffusion. Interestingly, low current, long duration treatments have been thought to not only drive more Dex into the tissue, but also deeper into the tissue when compared to high current, short duration treatments.² Unfortunately, these results were drawn from an in vitro study using agarose gel and cannot be directly compared to human skin.² In the second, the electrodes were left on the skin for the entire posttreatment period allowing for the drug to continue to passively diffuse through the skin. Although this was not tested in the current study, electro-osmosis initiated by the electrical current along with leaving the electrode on the skin could be a possible reason for seeing an increase in [Dex-total]

following the iontophoresis treatment. Future studies testing the same parameters without the electrode being left on will be required to test this theory.

Previous studies^{2,13} have produced conflicting theories concerning vasoconstriction and its effect on transdermal drug delivery. Gurney et al¹³ stated that vasoconstriction hinders diffusion of Dex-P, requiring more time for the drug to be found at deeper depths, while Anderson et al² stated that vasoconstriction promotes deeper drug penetration into the skin. While the vasoconstriction characteristics of ice may help prevent drug clearance, it is possible that its decrease in cell permeability may affect the concentration of the drug by altering one of the transportation pathways.

Dex-P delivered via iontophoresis is regularly used as a treatment for various musculoskeletal inflammatory conditions including tendinopathies.^{2,12,26,31} The tendons most frequently treated with Dex-P iontophoresis and their average depths are: Common extensors (1.2 mm), Achilles tendon (1.6 mm), and Patellar tendon (3.1 mm).⁵ Current research has yet to determine an ideal concentration of Dex to be delivered to the target tissue. It has yet to be determined whether a greater concentration of Dex would yield a more efficient clinical result, or if the ED₅₀ (75 ng/g) is sufficient for the preferred anti-inflammatory response.³¹ Dex has been shown to superficially form a depot^{2,27,30} in the stratum corneum (average depth of 22.6 μm in the forearm),⁹ and has been suggested to represent the highest concentration of the drug delivered.² Although we found a clinically relevant concentration of Dex when using an 80 mA·min dosage, surpassing the minimal standard ED₅₀, it was only recovered in 28% of the subjects at a depth of 3mm. It is possible that a clinically relevant [Dex] may be found in a higher percentage of subjects at a more superficial depth due to the formation of a drug depot at more shallow depths. This suggests that iontophoresis delivering Dex-P could allow for more consistent and effective results when used on tendons and other musculoskeletal inflammatory conditions that are more superficial to the skin's surface. Future research is needed in order to measure [Dex-P] at different depths using similar treatment parameters to identify true drug depot formation and if it would yield desired outcomes.

There were several limitations in this study. We only used healthy males and nonpregnant females between the ages of 18 and 40. We assume that similar Dex-total results would occur in other populations not represented in this study (eg, injured, younger, older). Different populations may have different skin hydration and status, which could alter iontophoresis delivery. Due to the depth choice of the microdialysis probes, we only measured [Dex-total] at an approximate depth of 3 mm. We did not determine [Dex-total] kinetics at deep tissue depths, which may be desired for some clinical pathologies. Our methods did not include a sham iontophoresis treatment. Due to this limitation, it is unknown if small amounts of Dex-P may have crossed into the skin without the current aiding delivery. We also used the forearm in assuming that the iontophoresis delivery and pharmacodynamics of Dex-P through tissue would be similar if done at common treatment sites over a tendon. It is possible that the physiological effects of the ice massage may have altered the diffusion gradient occurring through the semipermeable membrane of the microdialysis probes effecting the Dex-total concentration results. Due to potential variations in skin perfusion recordings from removing and reapplying the LDF probes while applying the ice treatment, we elected to measure a 1-minute skin perfusion baseline prior to beginning the iontophoresis treatment. Unfortunately, this does not represent a true baseline of skin perfusion pre-ice massage but allows us to understand the effects of the iontophoresis treatment on superficial skin perfusion.

CONCLUSION

Based upon the results of our study, we suggest that an ice massage not be performed prior to an iontophoresis treatment. The amount of [Dex-total] recovered, reaching the ED₅₀, indicates that a 4 mA current intensity for 20 minutes is a clinically relevant dose for an effective delivery of Dex-P to a tissue depth of 3 mm. We recovered all 3 of the components for Dex-total (Dex, Dex-met, and Dex-P), with a greater recovery of Dex-P than was previously seen with lower intensities. Future research should continue to investigate high current vs low current intensities for better clinical outcomes. Using a higher current intensity resulted in lower peak skin perfusion than a lower current intensity suggesting a possible threshold that must be met before drug clearance occurs.

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Figure 1. A cryocup was filled (with tap water) and frozen at approximately $-18^{\circ}C$ (0°F). The cryocup included a cold-retardant handle and contoured base for easier application.



Figure 2. Placement of laser Doppler flowmeter probes to measure skin perfusion during iontophoresis treatment. Laser Doppler flowmeter probes were placed inside the drug chamber (0 cm) and on the peripheral of the drug chamber (2 cm).



Figure 3. RP-HPLC standard curves of dexamethasone sodium phosphate (Dex-P) each point represents the mean area under the absorbance (239 nm) time curve (AUC).



Figure 4. Dex-total concentrations (Dex-total = Dexamethasone sodium phosphate + Dexamethasone + Dexamethasone-21-oic acid) between an ice and nonice group using a 4 mA intensity over an 80 mA·min iontophoresis dose (values are mean \pm 1 SEM).

*Indicates significant difference of Dex-total from baseline (P < 0.05).



Figure 5. Superficial skin perfusion response of 4 mA current intensity during an 80 mA min iontophoresis dose. Values are mean ± 1 SEM for 13 participants in each group.

	Concentration Frequency, Frequencies (Mean ± 1 SD)							
-	Dex-P		Dex		Dex-met			
Time	Ice	Nonice	Ice	Nonice	Ice	Nonice		
Treatment	4	7	—	_	4	4		
Ireatment	(0.235 ± 0.280)	(0.496 ± 0.496)			(2.070 ± 2.843)	(1.473 ± 0.893)		
Posttrootmont 0, 20 min	4	8	—	2	5	5		
Postireatment 0–20 mm	(0.105 ± 0.013)	(0.742 ± 1.423)		(2.266 ± 2.669)	(1.089 ± 0.831)	(1.407 ± 1.533)		
Destination and 20, 40 min	6	7	—	1	6	6		
Postireatment 20–40 mm	(0.547 ± 0.817)	(0.585 ± 0.555)		(0.428)	(2.139 ± 1.541)	(4.424 ± 2.987)		
Destination and 10, 60 min	4	5	2	3	5	6		
Posttreatment 40–60 min	(1.282 ± 2.349)	(0.837 ± 0.811)	(1.00 ± 0.394)	(1.327 ± 1.707)	(2.622 ± 1.231)	(6.163 ± 3.660)		

Table 1. Concentration frequencies for Dex-P, Dex, and Dex-met for ice and nonice groups during 20-min intervals.