

Dexamethasone Sodium Phosphate Penetration During Phonophoresis at 2 Ultrasound Frequencies

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Context: The effect of ultrasound frequency on phonophoresis drug delivery in humans is unknown.

Objective: To determine if a low (45-kHz) or high (1-MHz) frequency delivered a higher dexamethasone (Dex) concentration through the skin.

Design: Controlled laboratory study.

Setting: Laboratory.

Patients or Other Participants: A total of 40 healthy men between the ages of 18 and 45 years (age = 23.1 ± 2.6 years, height = 176.1 ± 7.2 cm, mass = 88.5 ± 19.4 kg, posterior calf subcutaneous thickness measured using musculoskeletal ultrasound imaging = 0.6 ± 0.2 cm).

Intervention(s): Participants were randomly assigned to 1 of 4 groups (ultrasound frequency at microdialysis probe depth): (1) 45-kHz frequency at 1 mm, (2) 45-kHz frequency at 4 mm, (3) 1-MHz frequency at 1 mm, or (4) 1-MHz frequency at 4 mm ($n = 10$ in each group). Three linear microdialysis probes were inserted at the desired tissue depth. We rubbed dexamethasone sodium phosphate (Dex-P) into the skin and then applied a 15-minute phonophoresis treatment.

Main Outcome Measure(s): Dialysate was collected during the treatment and 60 minutes posttreatment and analyzed for Dex-P, Dex, and the metabolite form of Dex. The sum of the 3 analytes was calculated as total dexamethasone (Dex-total), and differences between the 45-kHz and 1-MHz treatment groups were determined by a repeated-measures analysis of variance.

Results: At 1 mm, 3 (30%) participants in the 45-kHz and 4 (40%) participants in the 1-MHz group had measurable levels of Dex-P. Total dexamethasone increased after the treatment ceased, independent of ultrasound frequency ($P < .001$), with a trend of the 45-kHz treatment to produce a greater increase in drug concentration ($P = .006$). At 4 mm, 5 (50%) participants in the 45-kHz and 1 (10%) participant in the 1-MHz group had measurable levels of Dex-P. We observed no difference in Dex-total concentration between treatment groups at 4 mm ($P = .72$).

Conclusions: Phonophoresis provided a mechanism for Dex-total delivery at the 1- and 4-mm tissue depths. However, the effectiveness of the ultrasound frequencies varied between the 2 measured tissue depths.

Key Words: sonophoresis, microdialysis, anti-inflammatory

Key Points

- Total dexamethasone increased due to the phonophoresis treatment, regardless of the ultrasound frequency.
- Most transdermal drug delivery occurred after the treatment was concluded due to increased skin permeability.
- The rate of increase after the treatment was higher using the low-frequency device at the 1-mm depth but not at the 4-mm depth.
- The superficial difference in transdermal drug delivery with the low-frequency phonophoresis device may have been due to a variety of factors in addition to the frequency, including the use of a hydrogel dressing and different treatment variables.
- The low-frequency phonophoresis treatment provided transdermal drug delivery that was similar to or slightly better than the traditional 1-MHz treatment via a patch that is easy for patients and clinicians to use.

Transdermal drug delivery, the noninvasive administration of a drug through the skin at the site of treatment, has advantages over other drug-delivery methods. These advantages include decreased systemic drug concentration passing through vital organs, fewer potential side effects, and better patient compliance compared with injections.^{1,2}

Clinicians have used ultrasound as a mechanism to enhance transdermal drug delivery since the 1950s. *Phonophoresis* (usually associated with higher frequencies: 1 or 3 MHz) or *sonophoresis* (usually associated with lower frequencies: ≤ 45 kHz)³ is the enhancement of drugs or other compounds through the skin using ultrasound. It increases drug penetration through the skin during and after the treatment by cavitation, distorting structured lipids in

the stratum corneum.⁴ This disorientation in the epidermis increases the skin's permeability, allowing the topical drug, especially when it has a low molecular weight, to penetrate the dermis. A concentration gradient with high drug concentrations at the skin surface and low levels deeper in the tissues allows for the topical drug to slowly diffuse through the tissue's layers.⁵

Ultrasound frequency is a main factor in transdermal drug-delivery enhancement of phonophoresis. However, whether high- or low-frequency phonophoresis is more effective for transdermal drug delivery in humans is unknown. Investigators initially hypothesized that higher frequencies would lead to more ultrasound energy concentrated in the epidermis due to the inverse relationship with frequency and tissue absorption. Therefore, more substan-

tial transdermal drug-delivery enhancement would occur with higher frequencies.^{6,7} However, research^{8,9} has not supported this hypothesis. Mitragotri et al¹⁰ compared the enhancement ratios of 1-MHz and 20-kHz frequencies in vitro. They found that 1 MHz increased drug enhancement 4-fold but 20 kHz increased drug enhancement up to 1000-fold. The location of cavitation may factor into the increased differences in skin permeability. During high-frequency phonophoresis, cavitation occurs within the skin, whereas with low-frequency phonophoresis, cavitation occurs just above the skin.^{4,11,12} The lack of noninvasive research methods in humans has limited the ability to effectively test additional phonophoresis frequency factors.

Microdialysis is a minimally invasive technique for sampling local exogenous or endogenous compounds from the local tissues. Microdialysis probes, which have semipermeable membranes, are perfused with a solution, allowing equilibration between the solution and extracellular matrix.¹³ Given this equilibration, we can collect the analyte of interest and determine its concentration in the tissue.^{14,15} Using microdialysis, we can examine and refine phonophoresis factors to determine proper pharmacologically effective concentrations in the tissue. Researchers and clinicians can then perform randomized clinical trials to assess the clinical effectiveness of phonophoresis.

Over the past 2 decades, research has primarily focused on lower frequencies during phonophoresis. However, the implementation of low-frequency phonophoresis into clinical practice has been slow. Laboratory and animal investigators have demonstrated the effectiveness of low-frequency over high-frequency phonophoresis,¹² but no authors of recent randomized clinical trials have used low-frequency phonophoresis. The higher cavitation effect of low-frequency phonophoresis on disorganizing the stratum corneum was thought to irritate human skin.¹⁶ New phonophoresis technology, using a 45-kHz frequency, has been designed to limit skin irritation and improve low-frequency phonophoresis with an easy-to-use patch. This phonophoresis device uses a lower temporal average intensity than phonophoresis with a traditional ultrasound device.

We wanted to help refine phonophoresis factors to maximize clinical use in humans. Therefore, the purpose of our study was to answer the research question: Which phonophoresis frequency, 1 MHz or 45 kHz, delivers a higher dexamethasone (Dex) concentration [Dex], a commonly used anti-inflammatory drug, through the skin? Based on in vitro studies, we hypothesized that the lower-frequency phonophoresis would produce elevated drug concentrations in the tissue when measured via microdialysis.

METHODS

We used a randomized controlled laboratory study design to guide our data-collection procedures. All procedures took place in our Therapeutic Interventions Laboratory. The outcome measures for this study were dexamethasone sodium phosphate (Dex-P) concentration [Dex-P]; [Dex]; and dexamethasone-21-oic acid (Dex-met), the metabolite of Dex-P and Dex, concentration [Dex-met] in the tissue. All 3 of these analytes have been measured during transdermal drug delivery in human tissue using microdialysis methods.¹⁴ We also summed the concentrations of these analytes to give a total dexamethasone (Dex-total)

concentration [Dex-total] in the tissue. The independent variables for this study were phonophoresis frequency (low versus high frequency) and sample collection time.

Participants

We enrolled 40 healthy men between the ages of 18 and 45 years (age = 23.1 ± 2.6 years, height = 176.1 ± 7.2 cm, mass = 88.5 ± 19.4 kg, posterior calf subcutaneous thickness measured using musculoskeletal ultrasound imaging = 0.6 ± 0.2 cm). Dexamethasone sodium phosphate is a category C drug pertaining to its use during pregnancy. Given the unknown risks associated with pregnancy and lack of pregnancy testing in the procedures, we excluded females from this study. A screening questionnaire was used to determine whether participants were healthy and free of conditions associated with the contraindications for Dex-P and therapeutic ultrasound. Participants were not enrolled in the study if they had 1 of the following self-reported contraindications: systemic fungal infection, hypersensitivity to sulfites, diabetes, systemic or local infection, hypertension, elevated salt or water retention, tuberculosis, peptic ulcer, osteoporosis, vascular insufficiency or thrombophlebitis in the lower extremity, malignancy, presence of a pacemaker, or injury to the lower extremity within the 2 months before the study. All participants provided written informed consent, and this study was approved by the Institutional Review Board at Texas State University.

Instrumentation and Dialysate Analysis

We determined the [Dex-P], [Dex], and [Dex-met] in each microdialysis dialysate sample using high-performance liquid chromatography (HPLC) via a previously described method.¹⁷ We analyzed all 3 of these analytes due to their various structures and interactions with the tissues. Dexamethasone sodium phosphate is a prodrug that must be broken down in the tissue to its biologically active form, Dex. However, during transdermal drug delivery, Dex-P has been reported to metabolize quickly, leading to higher [Dex-met],¹⁴ which has a lower affinity for binding to the glucocorticoid receptor.¹⁸

A compact liquid chromatography system (model 1120; Agilent Technologies Inc, Santa Clara, CA) equipped with a 20- μ L sample loop, a variable-wavelength ultraviolet-visible detector set at 239 nm, and a liquid chromatography column (5- μ m particle size, 4.6 mm \times 250 mm; ZORBAX Eclipse XDB-C8; Agilent Technologies, Inc) was used to analyze the collected sample dialysate. Eluent A was an aqueous buffer containing 20 mM ammonium formate with the pH adjusted to 3.8 with formic acid, and eluent B was acetonitrile. The mobile phase was pumped at 1.0 mL/min with the isocratic elution set as 70% A and 30% B. Dexamethasone sodium phosphate, Dex, and Dex-met were measured at retention peaks of 4.3, 15.2, and 6.9 minutes, respectively. The lowest calibration concentration detectable for both Dex-P and Dex was 1 μ g/mL. The Dex-met was unavailable for direct calibrations. It was quantified using a combination of the Dex-P and Dex calibration curves. To determine if combining the Dex-P and Dex calibration curves was appropriate for the Dex-met analysis, we conducted an independent-samples *t* test and observed no difference between the calibration curves ($t_9 = -1.526$, $P = .16$).

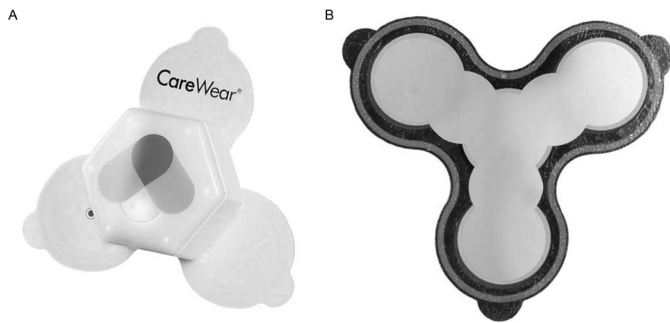


Figure 1. Low-frequency ultrasound patch (CareWear Corp, Reno, NV). **A,** Power controller attached to the patch. **B,** Transducer side of the patch. Treatment was delivered at 0.075 to 0.09 W/cm², using continuous mode and a frequency of 45 kHz.

Therapeutic Interventions

We used 2 different therapeutic ultrasound devices to administer the 45-kHz and 1-MHz phonophoresis treatments. We applied the 45-kHz treatment with the Carewear LFUS patch (Carewear Corp, Reno, NV; Figure 1). The patch is a broadband ultrasound transducer consisting of a flexible piezoelectric polymer printed on a 150- μ m positron emission tomography film that provides an average displacement across the field of 2.09 nm at 45 kHz, which is equivalent to a temporal average ultrasound intensity of 0.075 to 0.09 W/cm². The duty cycle was set to continuous mode. The manufacturer of the device programmed all settings, limiting our ability to make adjustments. The patch is attached to the user's skin surface using a hydrogel optimized for ultrasonic transmission, similar to the hydrogel used with electrical stimulation electrodes. We applied the treatment for 15 minutes over a surface area of 4.2 cm². The output of the Carewear LFUS patch falls within the safety limits of <0.10 W/cm² for a device that is affixed to the skin surface and with which the ultrasonic field is not continuously moved over the treatment area.

We administered the 1-MHz treatment with the Chattanooga Vectra Genisys Therapy System (DJO, LLC, Vista, CA). We used a 5-cm² transducer with a reported effective radiating area of 4.0 cm² and beam nonuniformity ratio of 5:1. We set the duty cycle to 50% and the spatial average intensity to 1.5 W/cm², making the temporal average ultrasound intensity 0.75 W/cm². We used a pulsed duty cycle based on previous research¹⁹ and its ability to limit the thermal effects of ultrasound.^{20,21} Large increases in cutaneous blood flow have been shown to cause drug washout from the local tissues.¹⁴ We applied the treatment for 15 minutes over a surface area of 8 cm² using a handheld transducer.

Based on the device technology available to us, there were more phonophoresis factor differences than just the frequency. Phonophoresis intensity and delivery methods were also different. We kept the treatment time similar for both interventions.

Procedures

Participants each made a single visit to the laboratory. At the beginning of the session, they provided informed consent, and the investigator (J.H.R.) screened them based on the inclusion and exclusion criteria. Participants were randomly assigned to 1 of 4 groups: (1) 45-kHz ultrasound

frequency with a 1-mm probe depth (n = 10), (2) 45-kHz ultrasound frequency with a 4-mm probe depth (n = 10), (3) 1-MHz ultrasound frequency with a 1-mm probe depth (n = 10), or (4) 1-MHz ultrasound frequency with a 4-mm probe depth (n = 10). Randomization was implemented using a number draw from a bag by the participant. Numbers on folded pieces of paper corresponded to the group assignment numbers. We included equal numbers in the bag to ensure that 10 participants were allocated to each group. The 1-mm probe depth was intended to determine superficial [Dex-P], [Dex], and [Dex-met] just below the skin surface. We chose the 4-mm probe depth because it corresponded with the depth below the skin surface of tendons commonly treated with phonophoresis (eg, Achilles tendon and common extensor tendons).²² In this laboratory study with no subjective outcome measures, neither the investigators (J.H.R. and A.M.H.) nor the participants were blinded during the data-collection procedures. The investigators who performed the HPLC analysis (A.R.K. and C.J.) were blinded to the participants' group assignments.

Three linear microdialysis probes with a molecular weight cutoff of 13 kDa were inserted into the left posterior leg at a depth indicated for the participant's randomly assigned group. Participants lay prone on a treatment table throughout the study. We visualized the largest girth of the posterior leg and made marks 5 cm apart where the guide needles were to enter and exit. The site on the posterior leg was cleansed with an iodine swab before the microdialysis probes were placed.

Using aseptic procedures, the investigator horizontally inserted 3 sterile 3.5-in, 25-gauge pediatric spinal-tap needles (model 4632V2; McKesson Corp, Golden Valley, MN) at the desired tissue depth. During the insertion of the needles, musculoskeletal ultrasound imaging was used to guide and verify the depth of probe placement. The actual depths for the 1- and 4-mm probe placements were 1.7 \pm 0.8 and 4.1 \pm 0.8 mm, respectively. The microdialysis probes were fed through each needle, and then the needles were removed, leaving the microdialysis probes in place in the posterior leg. Transparent film dressing and clear surgical tape were placed over the insertion and exit sites of the microdialysis probes to ensure cleanliness and prevent contamination of the portal sites with the drug during the treatment (Figure 2).

After the probes were inserted, the investigator cleansed the treatment area with isopropyl alcohol. Sterile saline (0.9% NaCl) was perfused through the microdialysis probes at 1.2 μ L/min for 60 minutes, and this time allowed the tissues to recover from the mild trauma that occurred during the guide-needle insertion. During the last 20 minutes of the recovery period, dialysate was collected and used as the baseline sample.

After the baseline measurement, the investigator rubbed 1 mL 0.4% liquid Dex-P into the skin over a 10-cm² treatment area. Marks were made on the posterior calf to indicate the treatment area. For the 45-kHz treatment, the investigator centered the transducer patch and hydrogel over the microdialysis probes, and the device was turned on for 15 minutes. For the 1-MHz treatment, 5 mL ultrasound gel was placed on the skin after the drug was rubbed in. The therapeutic ultrasound was turned on to a temporal average intensity of 0.75 W/cm² (50% duty cycle \times 1.5 W/cm²), and

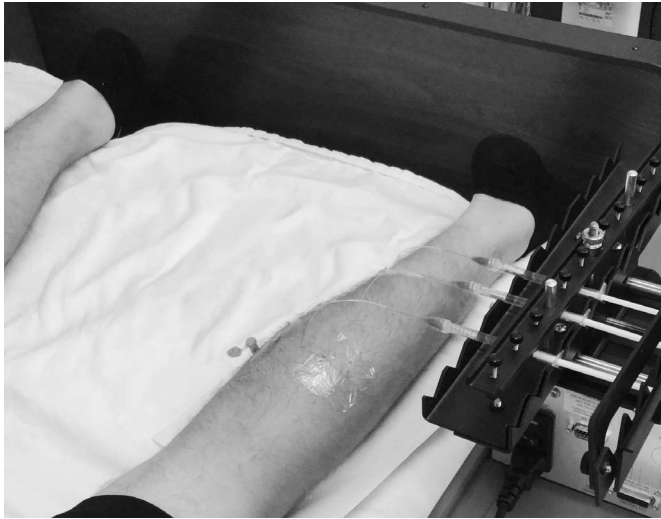


Figure 2. Microdialysis setup in the posterior calf. Three microdialysis probes were placed 1 or 4 mm below the surface of the skin.

the investigator linearly moved the transducer at approximately 4 cm/s. The treatment time was set for 15 minutes. The microdialysis probes were continually perfused with sterile saline throughout the treatment and posttreatment periods at 1.2 $\mu\text{L}/\text{min}$, and 1 dialysate sample was collected during this 15-minute treatment.

After the treatment, the investigator removed the therapeutic ultrasound device, and the remaining gel was wiped away with gauze. We did not clean the skin further until all procedures and posttreatment dialysate collection were finished. During the 60-minute posttreatment period, we collected dialysate samples every 15 minutes for a total of 4 posttreatment samples. Immediately after collection, dialysate samples were stored in a deep freezer at -80°C until they were analyzed using HPLC. After the 60-minute posttreatment period, the investigator removed the microdialysis probes and properly cleaned the insertion and exit sites.

Data and Statistical Analysis

We calculated frequencies and descriptive statistics for each Dex form (ie, Dex-P, Dex, and Dex-met) across the different phonophoresis frequencies and microdialysis probe depths. Before performing inferential statistical analyses, we calculated the sum of all forms of Dex in the dialysate samples and created a variable called Dex-total: $\text{Dex-total} = \text{Dex-P} + \text{Dex} + \text{Dex-met}$.

To calculate [Dex-total], zeros were inserted if no measurable [Dex-P], [Dex], or [Dex-met] was found using HPLC. Although small amounts of these analyte concentrations might have been present, the concentrations may have been below our instrument's detection limit.

Using Dex-total, we calculated a repeated-measures analysis of variance (ANOVA) to determine differences between the 45-kHz and 1-MHz treatments. A separate repeated-measures ANOVA was used at each microdialysis probe depth (1 and 4 mm). A repeated-measures ANOVA was used to determine if [Dex-total] was different between the 1- and 4-mm probe depths over the treatment and posttreatment times. For this analysis, treatment-group data

were pooled. The statistical powers were 0.554 for the 1-mm analysis and 0.086 for the 4-mm analysis. Given our small sample size and limited power, we were not able to appropriately run a $2 \times 2 \times 6$ (treatment group \times probe depth \times time) repeated-measures ANOVA to examine the 3-way interaction. All data analysis was completed using JMP Pro 13 (SAS Institute Inc, Cary, NC), and the α level was set at .05.

RESULTS

Dexamethasone Concentration

At the 1-mm microdialysis probe depth, we observed measurable levels of Dex-P in 3 (30%) and 4 (40%) of 10 participants using the 45-kHz and 1-MHz treatment frequencies, respectively. Of all the samples that had measurable levels of Dex-P, regardless of time, the mean concentrations were $1.231 \pm 1.895 \mu\text{g}/\text{mL}$ (95% confidence interval [CI] = $-1.123, 3.584 \mu\text{g}/\text{mL}$) and $3.567 \pm 3.510 \mu\text{g}/\text{mL}$ (95% CI = $1.337, 5.797 \mu\text{g}/\text{mL}$) for the 45-kHz and 1-MHz treatment frequencies, respectively. Dexamethasone was not detected in any of the participant samples. We recovered more Dex-met: all 10 (100%) participants had measurable levels using both treatment frequencies. Of all the samples that had measurable levels of Dex-met, regardless of time, the mean concentrations were $7.319 \pm 5.937 \mu\text{g}/\text{mL}$ (95% CI = $5.445, 9.193 \mu\text{g}/\text{mL}$) and $3.268 \pm 2.704 \mu\text{g}/\text{mL}$ (95% CI = $2.435, 4.100 \mu\text{g}/\text{mL}$) for the 45-kHz and 1-MHz treatment frequencies, respectively. The treatment frequencies and mean concentrations in the tissue for the 3 analytes are presented in Tables 1 and 2.

We observed a main effect of time, with an increase in [Dex-total] throughout treatment and posttreatment when averaged across treatment groups ($F_{5,90} = 23.62, P < .001$). For all times, we found a trend, which was not different, of the 45-kHz treatment delivering more Dex-total to the superficial tissues ($F_{1,18} = 3.08, P = .10$). A time \times treatment interaction was found ($F_{5,90} = 3.49, P = .006$). The increase in [Dex-total] in the tissue as a function of time is presented in Figure 3A.

At the 4-mm microdialysis probe depth, we observed measurable levels of Dex-P in 5 (50%) and 1 (10%) of 10 participants using the 45-kHz and 1-MHz treatment frequencies, respectively. Of all the samples that had measurable levels of Dex-P, regardless of time, the mean concentrations were $5.794 \pm 6.014 \mu\text{g}/\text{mL}$ (95% CI = $1.492, 10.097 \mu\text{g}/\text{mL}$) and $13.924 \pm 11.363 \mu\text{g}/\text{mL}$ (95% CI = $-14.303, 42.151 \mu\text{g}/\text{mL}$) for the 45-kHz and 1-MHz treatment frequencies, respectively. Dexamethasone was not detected in any of the participant samples. The Dex-met was measured in 6 (60%) and 4 (40%) of 10 participants using the 45-kHz and 1-MHz treatment frequencies, respectively. Regardless of time, the mean [Dex-met] was $2.091 \pm 1.216 \mu\text{g}/\text{mL}$ (95% CI = $1.565, 2.617 \mu\text{g}/\text{mL}$) and $2.783 \pm 3.052 \mu\text{g}/\text{mL}$ (95% CI = $1.021, 4.546 \mu\text{g}/\text{mL}$) for the 45-kHz and 1-MHz treatment frequencies, respectively. The treatment frequencies and mean concentrations in the tissue for the 3 analytes are presented in Tables 1 and 2.

A main effect of time was observed: more Dex-total was found immediately after the treatment than at baseline when averaged across treatment groups ($F_{5,90} = 2.378, P = .045$). We observed no main effect of treatment difference ($F_{1,19} = 0.132, P = .72$) and no time \times treatment interaction

Table 1. Dexamethasone Sodium Phosphate Concentrations in the Tissue

Time, min	Probe Depth			
	1 mm		4 mm	
	45 kHz	1 MHz	45 kHz	1 MHz
0				
Frequency ^a	0	0	0	0
Mean ± SD (95% CI)	NA	NA	NA	NA
15				
Frequency	1	2	5	1
Mean ± SD (95% CI)	0.029 ^b	7.293 ± 7.239 (-57.746, 72.331)	7.739 ± 8.033 (-2.236, 17.713)	20.703 ^b
30				
Frequency	3	4	2	1
Mean ± SD (95% CI)	1.909 ± 2.328 (-3.875, 7.694)	2.079 ± 2.273 (-1.539, 5.696)	4.384 ± 3.546 (-27.476, 36.245)	20.264 ^b
45				
Frequency	1	3	1	1
Mean ± SD (95% CI)	0.395 ^b	2.608 ± 2.683 (-4.058, 9.274)	6.719 ^b	0.806 ^b
60				
Frequency	0	2	2	0
Mean ± SD (95% CI)	NA	3.263 ± 3.115 (-24.723, 31.249)	1.882 ± 0.694 (-4.351, 8.115)	NA
75				
Frequency	0	1	0	0
Mean ± SD (95% CI)	NA	5.553 ^b	NA	NA

Abbreviations: CI, confidence interval; NA, not applicable; SD, standard deviation.

^a Number of participants who had measurable concentrations at each time.

^b The SD and 95% CI could not be calculated because only 1 participant had a measurable concentration.

($F_{5,90} = 0.467, P = .82$) for [Dex-total] between treatment groups. The change in tissue [Dex-total] as a function of time is presented in Figure 3B.

When pooling the treatment-group data, we observed a main effect of probe depth, with more [Dex-total] recovered by the 1-mm microdialysis probe ($4.079 \pm 4.873 \mu\text{g/mL}$; 95% CI = 3.199, 4.960 $\mu\text{g/mL}$) than by the 4-mm probe ($1.556 \pm 3.786 \mu\text{g/mL}$; 95% CI = 0.872, 2.241 $\mu\text{g/mL}$; $F_{1,38} = 9.110, P = .005$). A probe depth \times time interaction was also found; the [Dex-total] increased at the 1-mm compared with the 4-mm probe depth over the treatment and posttreatment times ($F_{5,190} = 12.351, P < .001$).

DISCUSSION

Various frequencies have been examined in phonophoresis laboratory experiments,^{23,24} but this has not altered phonophoresis factors in randomized clinical trials in humans. In most randomized clinical trials, 1 MHz has been used as the phonophoresis frequency of choice.²⁵⁻²⁹ Previous methods of drug detection in tissue have been unable to refine phonophoresis factors in human participants for use in clinical trials. We are the first to examine different phonophoresis frequency devices in vivo with humans. Just under the skin surface, more Dex-total passed through the skin with the low-frequency device. Most of this drug movement occurred after the end of the treatment.

Simultaneous application of the drug and ultrasound treatment enhances transdermal transport by increasing

diffusion through structural alteration of the skin. *Acoustic cavitation*, the formation and pulsation of gaseous bubbles under the ultrasound field, is the principal mechanism for ultrasound-induced skin alterations.^{30,31} It disrupts the lipid barrier matrix, creating and expanding imperfections in the stratum corneum.³² After the treatment and disruption of the skin's permeability, large increases in drug concentration of tissue often follow. Low-frequency phonophoresis produces a greater number of cavitation bubbles and more cavitation displacement at the skin surface.^{11,12,33} Therefore, low-frequency phonophoresis led to greater penetration of the [Dex-total] just under the skin's surface.

Given that most of the Dex-total delivery occurred through the skin's surface posttreatment, clinicians should use posttreatment practices to enhance further drug delivery. Saliba et al¹⁹ found greater [Dex] in the tissue when the drug was applied with an occlusive bandage before the phonophoresis treatment. An occlusive bandage applied posttreatment may also enhance transdermal drug delivery. The low-frequency device we used has a hydrogel covering that may also be used as a dressing to encourage drug absorption through the skin. The hydrogel needs to be engineered to ensure that a concentration gradient does not occur, as that would encourage movement of the drug into the hydrogel matrix. Future investigators need to determine the best posttreatment practices after phonophoresis and how long skin permeability is altered after different treatment variables.

The hydrogel on the low-frequency ultrasound device may have influenced tissue hydration. Increases in

Table 2. Dexamethasone-21-Oic Acid^a Concentration in the Tissue

Time, min	Probe Depth			
	1 mm		4 mm	
	45 kHz	1 MHz	45 kHz	1 MHz
0				
Frequency ^b	0	0	0	0
Mean ± SD (95% CI)	NA	NA	NA	NA
15				
Frequency	4	5	1	1
Mean ± SD (95% CI)	0.538 ± 0.235 (0.164, 0.912)	0.537 ± 0.286 (0.182, 0.892)	3.268 ^c	1.439 ^c
30				
Frequency	9	9	5	2
Mean ± SD (95% CI)	3.853 ± 2.862 (1.653, 6.054)	1.653 ± 0.869 (0.985, 2.321)	1.849 ± 1.211 (0.345, 3.352)	1.534 ± 1.275 (-9.921, 12.989)
45				
Frequency	10	10	5	3
Mean ± SD (95% CI)	7.234 ± 4.562 (3.971, 10.498)	3.307 ± 1.881 (1.962, 4.653)	1.890 ± 1.589 (-0.084, 3.863)	2.457 ± 2.704 (-4.260, 9.174)
60				
Frequency	8	10	6	4
Mean ± SD (95% CI)	10.830 ± 6.154 (5.685, 15.975)	4.111 ± 3.080 (1.908, 6.314)	2.308 ± 1.290 (0.954, 3.662)	3.137 ± 3.495 (-2.425, 8.699)
75				
Frequency	10	9	6	4
Mean ± SD (95% CI)	10.425 ± 6.706 (5.628, 15.222)	5.418 ± 3.042 (3.080, 7.757)	2.047 ± 1.103 (0.889, 3.205)	3.635 ± 4.456 (-3.454, 10.724)

Abbreviations: CI, confidence interval; NA, not applicable; SD, standard deviation.

^a The metabolite of dexamethasone and dexamethasone sodium phosphate.

^b Number of participants who had measurable concentrations at each time.

^c The SD and 95% CI could not be calculated because only 1 participant had a measurable concentration.

hydration increase skin permeability, leading to enhanced transdermal drug delivery.³⁴ An occlusive dressing allows the stratum corneum's hydration to equilibrate with the underlying tissues.³⁵ Although we did not directly measure tissue hydration, we hypothesized that tissue hydration increased due to the hydrogel acting as an occlusive bandage. Therefore, an environment was created for more drug penetration through the stratum corneum posttreatment, especially when using a water-based hydrogel.

Although we used minimally invasive techniques to measure in vivo drug concentrations in humans, our sample size was a limitation of this research. We opted for a small sample size to limit the associated risks to humans from microdialysis. This affected the statistical power of our study and the type of statistical analysis we could perform. Comparing frequencies and descriptive statistics may also help identify the influence of the phonophoresis devices on drug delivery (Tables 1 and 2). Most Dex-total was recovered as Dex-met. However, some participants had measurable levels of the prodrug Dex-P. The high-frequency phonophoresis group had more consistent results at the 1-mm tissue depth; however, at the 4-mm tissue depth immediately (minute 15) after the low-frequency phonophoresis treatment, more participants had measurable levels and a higher [Dex-P]. In addition to creating greater acoustic cavitation, the longer ultrasound wave may have initially pushed the drug deeper into the tissue during the treatment.

Overall, at the 4-mm depth, we observed little difference in [Dex-total] in the tissue between the treatment frequencies. Researchers³⁶ have hypothesized that drug concentration in the superficial tissue will diffuse to the deeper tissue depths over time. Our study was limited by the posttreatment collection time.

We found similar peak [Dex-total] in the tissue between phonophoresis and previously published concentrations using iontophoresis.¹⁴ However, the peak [Dex-total] in the tissue during iontophoresis occurred at the end of the treatment, and the concentration in the tissue immediately started to decline after the iontophoresis treatment due to tissue-perfusion washout.¹⁴ After phonophoresis, the drug concentration in the tissue continued to increase for up to at least 1 hour posttreatment. The combination of phonophoresis and other transdermal drug-delivery methods could have an additive or exponential effect. Future researchers should also determine if a combination of phonophoresis and iontophoresis would create better repulsion of the drug through the transient permeable skin barrier.

In bench-top experiments, investigators³³ have determined that a certain energy-density threshold is needed to create exponential changes in skin perfusion. As the frequency increases, the energy density needed increases. Given the nature of the therapeutic ultrasound devices we used, there were differences in spatial average intensity, duty cycle, and treatment area, creating different energy densities. The 45-kHz frequency treatment had a temporal average intensity approximately 10% (0.09 versus 0.75

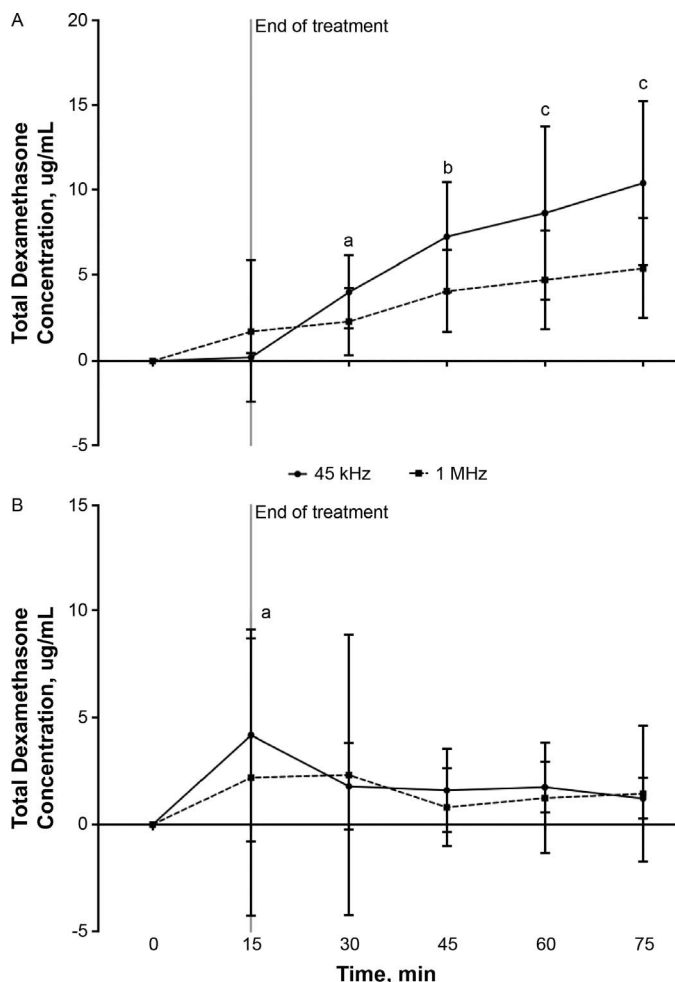


Figure 3. Total dexamethasone (Dex) concentration in the tissue (Dex sodium phosphate concentration + Dex concentration + Dex-21-oic acid concentration). **A**, at 1-mm microdialysis probe depth. **B**, at 4-mm microdialysis probe depth. Dex-21-oic acid is the metabolite of Dex and Dex sodium phosphate. ^a Different from 0 min ($P < .05$). ^b Different from 0 and 15 min ($P < .05$). ^c Different from 0, 15, 30, and 45 min ($P < .05$).

W/cm²) smaller than the 1-MHz treatment. However, the effectiveness of its drug delivery was slightly better or the same at all measurements. The lower energy density in the 45-kHz device allows for a safe therapeutic ultrasound patch, making the treatment easier for the patient and the clinician to use. A benefit of the lower-frequency device is that the clinician does not need to perform the therapeutic ultrasound treatment, and the patient could potentially be active during the treatment.

We collected actual [Dex-P], [Dex], and [Dex-met] in the tissue from healthy male participants. We assume that similar pharmacokinetics of Dex-P phonophoresis would occur between sexes. We also assume that a greater [Dex-total] in the tissue will lead to more positive clinical outcomes. Our HPLC's minimal detection limit for the analytes was greater than Dex's minimally effective dose. Therefore, all collected concentrations should have a biologic effect. As more phonophoresis factors and potential combination treatments are understood, researchers should determine the effectiveness of this transdermal drug-delivery technique and its factors in a clinical setting with patients who have pathologic conditions.

CONCLUSIONS

We observed an increase in Dex-total due to the phonophoresis treatment, regardless of the phonophoresis frequency. Most of the transdermal drug delivery occurred after the treatment was concluded due to increased skin permeability. The rate of increase posttreatment was higher using the low-frequency device superficially. However, this was not noted at the deeper 4-mm depth. The superficial difference in transdermal drug delivery using the low-frequency phonophoresis device may have been due to a variety of factors in addition to the frequency, including use of a hydrogel dressing and different treatment variables. A direct comparison of phonophoresis frequency alone was not performed due to the different technologies used for the treatments. The low-frequency phonophoresis treatment provided transdermal drug delivery that was similar to or slightly better than the traditional 1-MHz treatment, but it is a portable patch that is easy to use for patients and clinicians.

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