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Serum Dexamethasone Levels After Decadron Phonophoresis

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Objective: To determine serum levels of dexamethasone at several intervals after administration of Decadron (dexamethasone sodium phosphate) phonophoresis.

Design and Setting: This study was designed as a 2-factor analysis of variance with repeated measures on 1 factor (blood draws). Independent variables were group (gel/sham, gel/ultrasound, dexamethasone/sham, dexamethasone/ultrasound) and blood draws (pretreatment, posttreatment, 15 minutes, and 30 minutes). The dependent variable was the serum level of dexamethasone.

Subjects: Forty healthy college students (21 males, 19 females; mean age = 22 ± 1.3 years) with no known drug allergies or current medication use were randomly assigned to 1 of 4 treatment groups. The treatment site was the left forearm.

Measurements: After the pretreatment blood draw, a 10-minute ultrasound treatment was administered, followed by a posttreatment blood draw. Two additional blood draws followed at 15-minute intervals. A total of 4 serum samples (5 cc each) from each subject were centrifuged, and the pipetted serum was frozen for later analysis by double antibody radioimmunoassay.

Results: No significant amounts of serum dexamethasone were detected in 12 consecutive samples. Testing of additional samples was, therefore, discontinued.

Conclusions: Decadron phonophoresis as used in this experiment did not result in detectable serum levels of dexamethasone. More study is needed to validate the efficacy of Decadron phonophoresis on serum dexamethasone levels.

Key Words: corticosteroid, ultrasound, serum analysis

Phonophoresis uses ultrasound to enhance the absorption of topically applied drugs into underlying tissue, offering an alternative to injection or oral administration of medication. Previous researchers have reported contradictory findings as to the efficacy of this procedure.[1–5] There are 2 main factors that affect phonophoresis: the medium and the ultrasound administration.

For phonophoresis application, ultrasound gel is commonly mixed with anti-inflammatory medications, counterirritants, and anesthetics. If a medium is not a good transmitter of ultrasound energy, then it is impossible to achieve the desired phonophoretic effects. Many previous studies on phonophoresis,[1–3,6–10] as well as more current modality texts,[11–14] used or advocated the use of hydrocortisone preparations. In 1992, Cameron and Monroe[4] measured the transmission qualities of different phonophoretic media and determined that hydrocortisone preparations were poor transmitters of acoustical energy. Studies using hydrocortisone preparations as the medium, therefore, cannot be used to determine the efficacy of phonophoresis. It is our observation that many clinicians now use dexamethasone sodium phosphate, or Decadron (Merck & Co, Inc, West Point, PA), in their phonophoretic treatment as an alternative to hydrocortisone. Decadron is an injectable corticosteroid that, when mixed with ultrasound transmission gel, has been shown to be an effective transmitter of ultrasound energy.[15] Few studies, however, have been conducted to test the efficacy of Decadron phonophoresis.

The administration of phonophoresis, with specific reference to the ultrasound parameters, has been a second source of confusion in the literature. Wide ranges in the settings of frequency, intensity, duration, and mode have been used without any justification. Recent studies suggest continuous mode ultrasound[16–19] at a frequency of 1 MHz[19–21] and an intensity of 1.0 W/cm^2 for 10 minutes[7,22] as the parameters of choice for effective phonophoresis. We have, however, found no studies that have attempted to measure the presence of dexamethasone after Decadron phonophoresis using currently suggested parameters. Our purpose, therefore, was to determine how the administration of Decadron phonophoresis on continuous mode ultrasound at 1 MHz frequency and 1.0 W/cm^2 for 10 minutes affected serum levels of dexamethasone.

METHODS

This randomized, double-blind, clinical study measured the effect of 2 independent variables (factors) on 1 dependent variable. The dependent variable was serum dexamethasone level. The independent variables were group (gel/sham, gel/

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ultrasound, Decadron/sham, Decadron/ultrasound) and blood draws (pretreatment, posttreatment, 15 minutes, and 30 minutes).

Subjects

Forty college students (21 males, 19 females), of mean age 22 ± 1.3 years, initially participated in the study. Subjects were restricted to those not currently taking any medication, with no known drug allergies, and with no recent history of ecchymosis, infection, swelling, or injury to the treatment site. The left forearm was selected because of its proximity to the antecubital fossa with accompanying antecubital veins. The study was sanctioned by the Brigham Young University Institutional Review Board, which also approved the consent form signed by each subject.

Instruments

The ultrasound unit used was the Omnisound 3000 (Accelerated Care Plus-Physio Technology Inc, Topeka, KS). The generator operates at frequencies of either 1 or 3 MHz. The transducer surface area is 5 cm^2 with an effective radiating area (ERA) of 4.5 cm^2, meaning that nearly all of the transducer's surface transmits acoustic energy. The beam nonuniformity ratio of 1.8:1 allows treatment at higher average output intensities with reduced risk of localized tissue heating, periosteal damage, and transient cavitation.

We used Ultra Phonic ultrasound transmission gel (Pharmaceutical Innovations Inc, Newark, NJ) in the control groups and a mixture of Decadron and Ultra Phonic gel for the treatment groups. The Decadron/gel mixture was prepared by a licensed pharmacist who routinely mixes media for phonophoretic use. Both media were used at room temperature (25°C) to replicate the clinical setting. Blood samples were centrifuged using a Beckman Model TJ-6 Centrifuge (Beckman Instruments, Inc, Palo Alto, CA).

Procedures

Each subject cleaned the left forearm with soap and water and lay supine on a treatment table. To restrict all treatment areas to the same size, we placed a 2-ERA template 4 cm distal to the midline of the antebrachial fossa on the left forearm. Using sterile technique, a registered nurse inserted an 18-gauge angiocath into an antecubital fossa vein proximal to the treatment area. The angiocath remained in place during the entire session to allow for subsequent blood draws. A 5-cc pretreatment blood sample was drawn, and the line was flushed with 2 cc normal saline to maintain patency.

Using a table of random numbers, each subject was randomly assigned to 1 of 4 groups. Treatment A used 100% Ultra Phonic ultrasound transmission gel with sham ultrasound (the machine turned off). Treatment B subjects received continuous ultrasound at 1 MHz, 1.0 W/cm^2, for 10 minutes with gel. Treatment C subjects received sham ultrasound coupled with Decadron/gel mixture (16.65 mg dexamethasone/100 mL gel). Treatment D subjects received continuous ultrasound at 1 MHz, 1.0 W/cm^2, coupled with Decadron in gel. The treatment media were in coded, identical bottles, and the ultrasound administrator was unaware of the assigned treatment group. In each instance, 3 cc of the ultrasound couplant was applied to the treatment area, approximating 2 mm in thickness. The sound head was moved back and forth within the template at approximately 3–4 cm/s.

Immediately following the ultrasound treatment, saline was withdrawn from the catheterized line and discarded, and a 5-cc posttreatment blood sample was drawn. The line was flushed a second time with 2 cc normal saline. The third blood sample was obtained 15 minutes posttreatment using the same method. Thirty minutes after the treatment, saline was withdrawn for the last time, and the final 5-cc blood sample was taken. The angiocath was removed, the area cleansed with 70% isopropyl alcohol, and a bandage applied to the injection site.

All blood samples were centrifuged within 1 hour at a relative force of 2500*g* for 20 minutes. The serum was pipetted, placed in 5-cc aliquot tubes, and immediately frozen at −30°C for later analysis. All samples were coded to ensure that laboratory personnel were unaware of the experimental conditions for each sample.

Analysis

The samples were analyzed using a double antibody radioimmunoassay.[23] The assay consisted of 3 parts. The first step used a known amount of radioactive dexamethasone ("hot" dexamethasone) and paper chromatography to separate dexamethasone from other similar steroids in the blood, such as cortisol. The second used an antibody, developed in rabbits, that binds to both the hot and cold dexamethasone. The third step employed a second antibody, goat anti-rabbit γ-globulin, that binds to the first antibody and the accompanying bound dexamethasone. The remaining amount of hot dexamethasone is counted in a scintillation counter. The count of free hot dexamethasone is correlated with the amount of serum dexamethasone. The sensitivity of the assay to dexamethasone is 50 ng/dL (10^{-9} g/dL); hence, the assay would show if 0.5% of the 0.48 mg of dexamethasone placed on the skin penetrated to the serum.

Statistics

We initially planned to use a 2-way analysis of variance with repeated measures on 1 factor to analyze the data. There were 4 levels of the between-subjects factor (group): treatments A, B, C, and D. The second independent variable (multiple blood draws pretreatment, posttreatment, and at 15 and 30 minutes) was the repeated-measures or within-subjects factor. The dependent variable was the measured level of dexamethasone in the serum. The criterion for significance was set at $P < .05$.

RESULTS

The first 8 experimental samples analyzed (2 samples from treatment A subjects and 6 samples from treatment D subjects) showed no detectable level of serum dexamethasone (<50 ng/dL). Thus, due to the absence of serum dexamethasone in 8 consecutive treatment samples, testing of additional samples was discontinued by the laboratory. In order to verify the results of the analysis performed at our institution, 4 additional samples from treatment D subjects were analyzed by Endocrine Sciences, Inc (Calabasas Hills, CA). The values were reported as <30 ng/dL (10^{-9} g/dL). The sensitivity of the assays to dexamethasone in serum are 50 ng/dL and 30 ng/dL, respectively; hence, anything less than those values assumes an operational definition of 0. Because all of the samples were reported as less than the sensitivity of the measure, the need to perform additional analyses was deemed unnecessary.

DISCUSSION

To date, the question of the effectiveness of phonophoresis in driving corticosteroids into the body remains unanswered. Many of the previous studies suffered from flaws in methodology, in choice of media, or in use of ultrasound parameters that bring into question the researchers' conclusions concerning the efficacy of phonophoresis.

Griffin and colleagues[1,2,6–8] performed a series of studies investigating hydrocortisone phonophoresis in swine. The reported increase in cortisol levels in skeletal and nervous tissue was not necessarily a result of the treatment, because the skin surface was burned[1,8] and the animals were manually restrained before they were anesthetized.[1,2] Either of these conditions could account for an increase in cortisol levels, since a disruption in the skin surface would allow for increased cortisol penetration and, as speculated by the authors,[7] the stress of the manual restraint would cause an outpouring of cortisol from the adrenal cortex.

Three later studies using the human forearm as a treatment site supplied additional information, yet still suffered from methodologic problems. Benson et al[16] used a medium found to be transmissive to ultrasound; however, the back-diffusion technique for measuring drug absorption used for the first time in this study was reported to be an inefficient method of measurement. Oziomek et al[17] and Bare et al[5] attempted to measure serum levels of trolamine salicylate (Myoflex, Bayer Inc, Toronto, Ontario, Canada) and 10% hydrocortisone, respectively. Both of these media, however, were identified by Cameron and Monroe[4] as poor transmitters of ultrasound.

Many of these recent studies seem to show that phonophoresis is not effective. Because of flaws in methodology, however, they cannot be used to evaluate phonophoresis efficacy. One recent study on mini Yucatan pigs, conducted by Byl et al in 1993,[15] lends credit to phonophoresis. Collagen deposition was measured in response to the following treatments. Hydrocortisone acetate and dexamethasone were applied by rubbing, injecting, or sonating (ultrasounding) the drug mixed with ultrasound gel (1 MHz, 1.5 W/cm^2, for 5 minutes). They tested the ultrasound transmission quality of the sonating mixtures and found that the dexamethasone mixture ranged from 95% to 98%, whereas the hydrocortisone mixture was recorded at less than 1%. They reported significant effects of the sonated dexamethasone, the injected dexamethasone, and the injected hydrocortisone, but not the sonated hydrocortisone. Because of its excellent transmission quality and seemingly positive outcome, we used a similar mixture of Decadron and gel.

One additional study using Decadron produced favorable results. Relief from trigger-point pain was reported in 88% of patients treated with Decadron and lidocaine phonophoresis, whereas a control group receiving the same medium via sham ultrasound reported only 23% relief.[24] It cannot be concluded, however, whether the results were due to the Decadron or lidocaine alone or the combination of the 2.

Our research employed a medium known to transmit ultrasound well and employed appropriate ultrasound parameters, yet our results did not support the use of phonophoresis. There are several possible reasons.

Phonophoresis may be ineffective, and ultrasound may not enhance the absorption of topically applied drugs into underlying tissue. We are not convinced that this is the case. In the Byl et al study[15] on mini Yucatan pigs using 2 corticosteroids (hydrocortisone acetate and dexamethasone), the fact that there was a decrease in collagen deposition with the sonated dexamethasone indicates its presence. There are several possibilities as to why we did not detect it.

It is possible that, after the drug is absorbed through the skin, it is sequestered in the subcutaneous tissue. Therefore, it would not be present in serum until a later time, since it is slowly released. A second finding from Byl et al[15] supports this idea. Reduced collagen deposition after dexamethasone phonophoresis was measured in the subcutaneous tissue, but not in the submuscular or subtendinous tissue. The inclusion of a later blood draw, perhaps 12 to 24 hours after the treatment, might have been appropriate to investigate the possibility of slow release of the drug from the subcutaneous tissue. Unfortunately, an error in the analysis of our pilot data led us to believe that the levels of serum dexamethasone peaked 15 minutes after the administration of Decadron phonophoresis. Therefore, our blood samples were drawn at pretreatment, posttreatment, 15 minutes, and 30 minutes.

It may be that the injectable form of Decadron we administered does not show up in the assays we used. After an extensive review of available assays, we found no assay specific to Decadron. However, we did identify a double antibody radioimmunoassay to test for serum dexamethasone.[23] Since dexamethasone is the active ingredient of Decadron, we chose this assay to test the absorption of Decadron. Although the analysis of pilot data appeared to demonstrate the effectiveness of the testing procedure and the accuracy of the assay when used with Decadron, analysis of subsequent samples showed no presence of serum dexamethasone. In order to examine the discrepancy between the pilot data and the first 8

samples tested, we sent 4 additional samples to an outside laboratory. The assay that the outside laboratory performed differed only in that it did not use the second antibody, but rather ammonium sulfate precipitation, to separate the bound and free dexamethasone. That laboratory also reported no presence of serum dexamethasone. On additional review of the pilot data, we found errors in the analysis that resulted in the false-positive appearance of serum dexamethasone in the pilot data. Both radioimmunoassays are generally used to analyze serum after an oral dose of dexamethasone. In injectable Decadron, a phosphate group is attached to the 21-carbon, which may interfere with the binding of the antibody in the first step of the assay. If this is the case, neither assay would recognize the phosphorylated dexamethasone.

CONCLUSIONS

Our study showed no levels of dexamethasone in serum after Decadron phonophoresis in 12 samples. We advocate the need for further research using an appropriate medium and appropriate ultrasound parameters before a conclusion can be drawn concerning the efficacy of phonophoresis. Future research should employ the following: tissue biopsy of the subcutaneous tissue, use of a different form of dexamethasone with one of the described assays, or use of a different radioimmunoassay specific to Decadron.

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