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The Pre-Application of Hydrocortisone Cream and Its Effect on Transdermal Drug Delivery by Phonophoresis

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Abstract

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Context: Transdermal delivery of hydrocortisone by phonophoresis is used for the treatment of musculoskeletal conditions. Research shows hydrocortisone and other white or opaque topical preparations transmit ultrasound energy poorly. Effective transmission of ultrasound is important in phonophoresis. **Main Outcome measured:** Samples of subcutaneous interstitial fluid were collected during and for 20 minutes following phonophoresis treatment. Cortisol concentrations were analyzed by an enzyme linked immune-assay (ELISA) test. **Objective:** Determine the subcutaneous cortisol concentration after two different phonophoresis treatments using a 2.5% hydrocortisone preparation. **Design:** Randomized design in which 22 healthy participants were assigned to receive a phonophoresis treatment where: 1) hydrocortisone cream was rubbed in completely prior to phonophoresis or 2) hydrocortisone powder was compounded with an ultrasound coupling gel. **Test Subjects:** 22 healthy individuals were recruited: 13 females with a mean age of 21 years and 9 males with a mean age of 21.8 years. **Intervention:** Phonophoresis consisted of pulsed ultrasound at 1 MHz, 1.0 w/cm^2, and a 50% duty cycle. The treatment duration was 10 minutes and was localized over the distal gastrocnemius muscle. **Results:** We observed no significant difference in subcutaneous cortisol concentration between the two phonophoresis treatments ($p=0.05$). Also no significant difference was detected between pre and post-treatment cortisol levels within each individual treatment group. **Conclusions:** Our data indicates that completely rubbing a topical hydrocortisone application into the skin prior to placement of ultrasound gel does not result in increased transdermal delivery of cortisol when compared with the use of a compound of ultrasound gel and hydrocortisone powder applied topically to the skin.

Key words: phonophoresis, hydrocortisone, microdialysis

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Introduction

When localized delivery of medication is desired for the treatment of musculoskeletal trauma, injection using a hypodermic needle is the most commonly used method.[1] The use of hypodermic needles, however, can result in pain or possible infection, thus decreasing patient compliance to the procedure. Transdermal drug delivery is the administration of therapeutic agents through intact skin.[2] Transdermal drug delivery presents an alternative means of local drug delivery. Phonophoresis is a form of transdermal drug delivery, where therapeutic ultrasound is used to increase the transcutaneous transmission of drugs. Phonophoresis can be used to introduce medication locally to a specific area without painful injections. Therapeutic ultrasound is thought to augment transdermal drug delivery by modifying the permeability characteristics of the skin.[3] Increased skin permeability resulting from ultrasound has been attributed to a phenomenon called cavitation. Cavitation refers to gas bubbles within the tissue which are made to oscillate as a result of the penetrating ultrasound energy.[3, 4] The oscillation of these bubbles near the skin provokes disorganization through the lipid layers of the stratum corneum. This disorganization is thought to create aqueous channels through the stratum corneum and increase the permeability of the skin.[4, 5]

During phonophoresis, one of two pre-treatment protocols is commonly used.[6] In the first protocol a topical medication alone is applied to the skin. This topical medication serves as the ultrasound coupling agent. The second pre-treatment protocol used in phonophoresis involves mixing the topical medication into a substance that is intended for use as an ultrasound coupling agent. Some topically applied pharmaceuticals; however, which are commonly used for phonophoresis have shown poor conductivity such as those that might be opaque or thick in consistancy.[6] Cameron et al. also showed that adding a medium that transmits poorly in equal

parts to a medium that transmits ultrasound energy well did not improve transmission.[6] A coupling medium should allow ultrasonic energy to enter the target tissue at the desired intensity. Any attenuation of the ultrasound energy may decrease the effectiveness of phonophoresis. Because of this, both of the above mentioned methods may compromise the effectiveness of some phonophoresis treatments.

Phonophoresis is both non-invasive and allows for the local introduction of medication.[1] An objective method of data collection was selected that would also fit those two criteria. Microdialysis probes were utilized to collect samples of interstitial fluid local to the treatment site which was used in quantifying drug delivery. The efficacy of pharmaceuticals is often based on an association between observed affects and drug concentrations in the blood plasma.[7, 8] Concentrations of drug molecules can differ significantly between blood and the tissues.[9] Researchers have shown that only drug molecules in the target tissue are responsible for the efficacy of a drug.[10, 11] Unlike samples gathered through a blood draw; samples collected through microdialysis probes come from interstitial fluid within the target tissue as opposed to circulating blood plasma. Microdialysis is currently the most appropriate sampling technique that provides for the analysis of drug molecules within a tissue.[8, 12]

The purpose of this study was to evaluate the effectiveness of phonophoresis treatments in which hydrocortisone cream is rubbed completely into the skin prior to the phonophoresis treatment using an ultrasound gel couplant. No researchers have specifically addressed the efficacy of thoroughly applying a thick, white topical medication similar to hydrocortisone preparations into the skin before the ultrasound gel is applied on top of the skin.

Methods

Study Design

The 2x2 design of this study includes 2 treatment groups and 2 covariates. The treatment groups are as follows: 1) a 2.5% hydrocortisone cream was rubbed into the skin for 5 minutes followed by application of an ultrasound gel and phonophoresis treatment, and 2) a 2.5% hydrocortisone powder compounded in ultrasound gel was applied to the skin in preparation for a phonophoresis treatment.

The depth of the microdialysis probe as well as the test subject's pre-treatment cortisol levels were measured and used as covariates. Volunteers with recent or current injury to the lower extremity, history of general illness or decreased sensation to lower extremities were excluded from participation. Success of the phonophoresis treatment or transdermal drug delivery was assessed objectively by measuring the difference in cortisol concentrations in the dialysate collected from interstitial fluid both prior to and following the treatment.

Subjects

There were 22 healthy individuals recruited as subjects and tested during this study: 13 females (mean age of 21 years) and 9 males (mean age of 21.8 years.) All subjects were college students from Brigham Young University. Subjects received treatment from one of two treatment groups to which they were randomly assigned. Prior to participation in this study all volunteers were required to read and sign the Institutional Review Board approved consent form.

Instruments

Ultrasound Unit: The ultrasound treatment was applied with an Omnisound 3000 Ultrasound device (Accelerated Care Plus, Reno, NV) with a 7.2 cm diameter sound head and a 5 cm^2 crystal at an intensity of 1.0 W/cm^2 with a 50% duty cycle.

Aquasonic 100 water-soluble hypoallergenic ultrasound transmission gel (Parker Laboratories, Inc, Fairfield, NJ) was used as the ultrasound coupling agent in the pre-application of topical medication treatment group.

A hydrocortisone cream with a 2.5% concentration (Fougera A division of Nycomed US Inc. Melville, New York 11747) was used.

Hydrocortisone powder with a 2.5% concentration from Medisca Chemical, Plattsburgh NY for use in this study. This compounding of the U.S gel and Hydrocortisone powder was prepared by a pharmacist at the Brigham Young University Student Health Center Pharmacy and used in group #2.

A Model LogiQ 5e, Doppler imaging ultrasound (General Electric Company, Fairfield, CT) was used to measure the depth of the microdialysis probe subcutaneously following insertion.

A cortisol high sensitivity ELISA kit (catalog #CO194S) from CalBioTech (A Life Sciences Company, 10461 Austin Drive suite G, Spring Valley, CA USA.) was used for the chemical analysis of the collected samples.

Tissue Perfusion

Sterile saline was perfused through the microdialysis probes using a Harvard Apparatus PHD 2000 Programmable Infusion Pump (Harvard Apparatus, Holliston, MA).

Probe construction

The microdialysis probes used in this study were constructed in a similar manner to those used in previous research involving transdermal drug delivery.[13] One difference was employed during the construction of the microdialysis probes for this study. In order to approximate clinical protocols for ultrasound use in our study a template was made to ensure that the ultrasound treatment performed on each test subject covered an area no larger than twice the diameter of the sound head on our therapeutic ultrasound unit. To more closely match the length of the treatment area in this study the hollow-fiber section in the microdialysis probes was increased from 2.5 cm to 5 cm.

Probe placement

The treatment site in this study was located on a skin site over the musculotendonous junction of the Gastrocnemius muscle and the Achilles tendon on the left leg of each research subject. (See Figure 1) This area was cleansed and sterilized with a Provadone swab. The microdialysis probe was then inserted subcutaneously. A 27-gauge needle was used as a guide cannula for microdialysis probe placement. The guide cannula was inserted under the skin in-line with the long axis of the gastrocnemius muscle. Early clinical trials in humans showed microdialysis probes successful in collecting samples in the subcutaneous adipose tissue.[14]

The cannula entrance and exit sites in the skin were separated by approximately 6.0 cm. This length was meant to approximate the size of the template which was used to create a uniform treatment area for each ultrasound treatment. Following insertion of the needle or guide cannula its depth and the depth at which the probe would rest throughout the study protocol was measured and recorded with a Doppler imaging ultrasound.

Mean probe depth was 0.3118 cm ± 0.09. The microdialysis probe was threaded through the inside of the guide cannula which was when removed.

To ensure that the cortisol detected in the interstitial fluid passed transdermally and to avoid contamination of the samples of dialysate collected, the entrance and exit portals for the microdialysis probes were sealed to the skin using a Tegaderm patch.

Drug Delivery

After placement, the microdialysis probes were perfused with 0.9% sterile saline at a rate of 10µl/min for 60 minutes using an infusion pump.[13] The purpose of this period was to allow the tissues to recover from the trauma of the needle insertion and for any elevated physiologic or inflammatory responses to return to base-line. At minute 60 of the recovery period the infusion rate was adjusted to 2 µl/min and a sample was collected for 30 minutes to determine pre-treatment tissue cortisol levels. The perfusion rate was maintained at 2 µl/min for the remainder of the study protocol.

Prior to the ultrasound treatment the test subjects in each of the two treatment groups received a different preparation. Two milliliters of the 2.5% hydrocortisone cream was rubbed into the skin of the 11 test subjects in group one for 5 minutes. Following those 5 minutes the excess cream was removed and 3 ml of water-based ultrasound gel was then applied to the skin. Test group two had 5 ml of a prepared compound of 2.5% hydrocortisone powder and ultrasound gel applied to the treatment site. Both of these were followed by a 10 minute ultrasound treatment to complete the phonophoresis. During the ultrasound treatment dialysate from the subcutaneous microdialysis probe was collected from the treatment site for analysis. The parameters for the ultrasound treatment were the same for all study subjects: 1 MHz, 1.0 w/cm^2, 50% pulsed duty cycle. A pre-fabricated template was placed on the skin of each research subject

to ensure consistency in the ultrasound treatment and confine the treatment area to 2 times the size of the ultrasound head.

At the conclusion of the 10 minute phonophoresis treatment, any remaining water-based gel (in test group 1) or combination of water based gel and hydrocortisone powder (in test group 2) was carefully removed from the treatment site. Subjects remained in a prone position for an additional 20 minutes while dialysate collection continued. Thirty minutes after the ultrasound treatment was initiated the saline perfusion was discontinued and the dialysate collected. Following the 120 minutes of the designed study protocol the ultrasound template and the subcutaneous microdialysis probe was removed. The portal sites was again cleansed with alcohol and covered. The collection vials were removed, labeled, and stored in a freezer at -20° C for later analysis.

Statistical Analysis

The data collected from each group was analyzed using an ANCOVA to determine whether one treatment method yielded a greater change in post-treatment cortisol levels. Analysis was also done to compare the pre-treatment and post-treatment cortisol concentrations in each individual group to determine the effectiveness of the phonophoresis treatment.

The measured depth of the microdialysis probes and pre-treatment dialysate cortisol concentrations were used as covariates. Cortisol levels fluctuate between individuals and even throughout the day in the same individual in response to many variables. Because the difference in cortisol levels within the body of each test subject the pre-treatment cortisol levels for each individual were recorded and used as another covariate in the data analysis. For our statistical analysis the level of significance was set at $P \leq 0.05$.

Results

In treatment group #1 the change in dialysate cortisol concentration after the phonophoresis was 13.2± 67.7 ng/ml; not significantly different from zero (t19=.96, p=.35). In treatment group #2 the change in dialysate cortisol concentration after the phonophoresis was -15.7 ± 30.3 ng/ml, also was not significantly different from zero (t19= -1.14, p=.27). Overall there were no significant differences in post-treatment dialysate cortisol levels between treatment groups. Nor was there a significant difference between pre and post-treatment dialysate cortisol concentrations in either of the individual treatment groups.

The pre-treatment, post-treatment, and change in dialysate cortisol concentrations were similar in both treatment groups (See Table 1). However, the pre-treatment dialysate cortisol levels significantly influenced the magnitude of the change in dialysate cortisol levels (P=0212.) Specifically, when higher values for pre-treatment dialysis cortisol concentration were seen, a smaller increase in post-treatment dialysis cortisol concentration could be expected. Microdialysis probe depth was not found to contribute significantly to the magnitude of the change in dialysate cortisol levels (p = 0.9735), and was subsequently removed from the analysis model.

Discussion

Research has found the hydrocortisone to be a poor conductor of ultrasound energy both in the common OTC preparation which is thick and white and when mixed in solution with ultrasound couplant gel.[6] This led, in part, to our hypothesis and study design to first topically apply the hydrocortisone prior to the application of the ultrasound gel so that only the coupling gel was present between the soundhead and the skin during the ultrasound treatment.

The success of phonophoresis has been reported by researchers for the treatment of musculoskeletal conditions, although some of these studies based their conclusion on subjective data. [13, 14-17] This subjective data may be collected through an increase in function or a decrease in pain communicated by a visual analogue scale. The term Clinical Evaluation also has been used to describe methods by which subjective data have been gathered in studies which reflect positively on phonophoresis. In 2005 Pribicivic and Pollard reported the success of a multi-modal treatment for shoulder impingement which involved a phonophoresis treatment with 1 percent hydrocortisone cream. [18] In other research involving phonophoresis, chemical analysis of either blood samples or tissue biopsies was used as a means of collecting more objective data. Older studies where chemical analysis was used to gather data reported successful phonophoresis treatments. [19, 20] More recent studies where a chemical analysis was employed seem to trend toward a negative outcome in the evaluation of phonophoresis. [21, 22, 23]

In our study a chemical analysis was used to evaluate the cortisol concentrations found in samples of interstitial fluid. By this method, the success of the phonophoresis or transdermal drug delivery of cortisol could be objectively evaluated. The results of our study, when compared to other research.[21, 22, 23] support the hypothesis that phonophoresis and hydrocortisone cream is a poor method for delivery of cortisol to underlying tissue.

No significant difference was found in the change in dialysate cortisol concentration between the two treatment groups. These results do not support the idea that rubbing a hydrocortisone cream into the skin prior to the application of ultrasound gel is more effective for phonophoresis than simply mixing hydrocortisone cream with ultrasound gel. Also, at the beginning of this study the assumption was made that the phonophoresis treatments would be effective in the transdermal delivery of cortisol. Because no significant change was seen

between the pre and post treatment dialysate cortisol levels in either of the two treatment groups the results of this study do not support this assumption that phonophoresis effective in the transdermal delivery of the hydrocortisone.

Hydrocortisone was the topical medication used in this study because of its thick, white or opaque preparation. Cortisol is a naturally occurring substance in the body and fluctuations of this chemical inside the human body as a result of a multidude of factors may have limited our ability to collect a sample and accurately measure a baseline concentration of tissue cortisol.

"Although microdialysis is a minimal invasive technique it is obvious that insertion of the probe will increase the local blood flow." [24] In 1998 Peterson reported an increase in histamine levels induced by the trauma caused by insertion of a microdialysis probe.[25] In our study, as has previously been done[26, 27] a recovery period (60 minutes) after the insertion of the probe designed to allow the local tissue to return to a homeostasis after the trauma of the probe insertion. One potential limitation of our study may be that the recovery time after the insertion of the cannula needle was insufficient to allow the underlying tissue to return to a normal physiologic state. Some of the base-line cortisol measurements in our study were greater than those cortisol measurements taken post-treatment. The elevated pre-treatment tissue cortisol levels may indicate the presence of local "unresolved" trauma.

In a study by Lee, microdialysis probes were used for data collection in the dermis. The microdialysis probes were inserted at a depth of approximately 3 mm. The recovery period after the insertion of the probe and before samples were taken was 150 minutes in length. Skin blood flow directly over the microdialysis probe was measured to ensure that the initial inflammatory or physiologic response to the placement of the probe had returned to baseline.[28] An increase of both local blood flow [24] and histamine[25] has been addressed in previous research; as a result of

the insertion of microdialysis probes in human skin. If a correlation between the two could be shown then a more appropriate recovery time could possibly be verified during a study protocol by using superficial blood flow as an index of recovery from the insertion trauma.

In 2011 Yeager explored the serum cortisol concentrations required to produce a systemic anti-inflammatory effect in post-surgical (cardiopulmonary bypass) patients as characterized by the concentration of plasma IL-6.[29] Yeager describes 'normal' serum cortisol concentrations in the range 15–20 ug/dl. It was found that an increase to a level of 35–45 ug/dl, a nominal 228% increase from 'normal' serum cortisol levels, produced an anti-inflammatory effect. Pre-treatment (normal) tissue cortisol concentrations measured in this study were 4.57 ug/dl and 4.16 ug/dl in Group 1 and Group 2, respectively, which are roughly one-third to one-fourth the normal measured blood serum concentrations reported by Yeager.[29] Caution needs to be exercised when drawing parallels between serum cortisol levels and tissue concentrations. If we can extrapolate from serum cortisol levels of the Yeager investigation to tissue cortisol levels measured in our study, we would expect that an approximate two-fold increase tissue cortisol level would yield any anti-inflammatory response. Our study produced a 29% increase in tissue cortisol levels for Group 1, and an unexpected 35% decrease in cortisol levels for Group 2. Thus, extrapolating from the Yeager study, neither of the two pre-treatment protocols for topical cortisone application by phonophoresis investigated here appears to produce increases in tissue cortisol concentrations that would result in an anti-inflammatory response. In fact, the average increase in cortisol concentration in our study (29% in Group 1) is only roughly one-eighth the increase required in serum cortisol concentration (228%) suggested by Yeager to produce an anti-inflammatory effect.[29]

Future research using microdialysis probes to collect interstitial fluid to find a base-line or pre-treatment measurement of cortisol should consider an increased recovery time after insertion of the cannula needle and microdialysis probe. The insult to the skin resulting from the insertion of the catheter needle may result in increased blood flow as well as other chemical mediators produced and released by the body. An increase in recovery time after insertion of a cannula needle may not be as important when using chemicals that are not naturally occurring in the body. Verification of blood flow associated with any physiologic inflammatory response due to the insertion of a microdialysis probe into the skin should be investigated in future studies.

Clinical Implications

The purpose of this study was to evaluate the effectiveness of a phonophoresis treatment with hydrocortisone cream by first rubbing the cream completely into the skin prior to the application of ultrasound through a gel couplant. Our data do not support the hypothesis that our modified phonophoresis protocol, rubbing the hydrocortisone cream into the skin prior to the application of ultrasound gel, would increase the effectiveness of the phonophoresis. Further the data collected did not support the assumption that the phonophoresis protocol used in this study would be effective in transdermal delivery of cortisol.

This study was designed with clinical protocols in mind. One study found that substances used in phonophoresis that had a thick white preparation, including hydrocortisone, conducted ultrasound energy poorly.[6] It was also found that combining these same substances in equal parts with a substance that has been shown to conduct ultrasound energy well did not result in an increase in conductivity when compared with water.[6] It was based off of this that a change in pre-treatment protocol was suggested where the topical hydrocortisone would be rubbed into the skin with any excess being removed prior to the application of ultrasound couplant gel so that

only a substance shown to conduct ultrasound energy well would be present between the sound-head and the skin during the ultrasound portion of the phonophoresis. Although the theory behind the suggested phonophoresis protocol technique adjustment seems sound no change in clinical protocols can be suggested based off of this study. In addition the use of hydrocortisone in phonophoresis should be reconsidered as a therapeutic modality for the treatment of musculoskeletal conditions.

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Table 1.

Study Measurement Values

Treatment Group	Probe Depth cm	Pre-Tx Dialysate Cortisol ng/ml	Post-Tx Dialysate Cortisol ng/ml	Change in Dialysate Cortisol ng/ml
#1 (HC)	0.320 ± 0.095	45.7 ± 48.7	57.9 ± 59.9	13.2 ± 67.7
#2 (Comp)	0.300 ± 0.093	41.6 ± 54.2	27.0 ± 39.4	-15.7 ± 30.3
Difference	0.02±0.002	-0.5 ± 0.2*		28.8 ± 19.5

Values represent the mean ± 1 SD for 11 subjects in each group.

Figure 1: Guide Cannula Needle

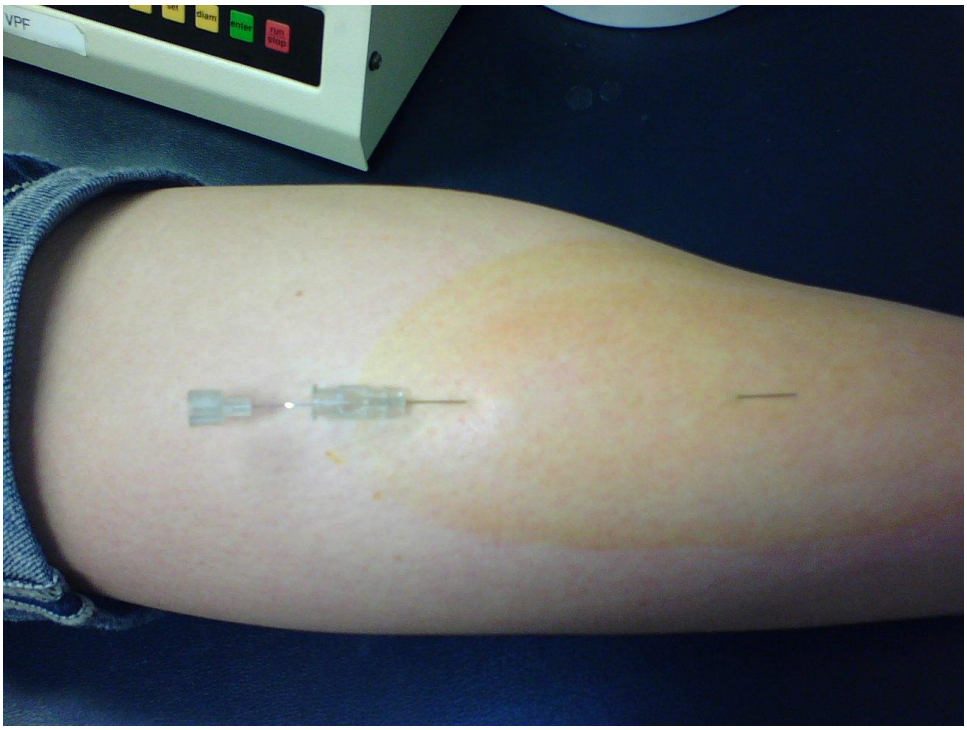

Figure 2: Doppler Ultrasound Measuring Probe Depth

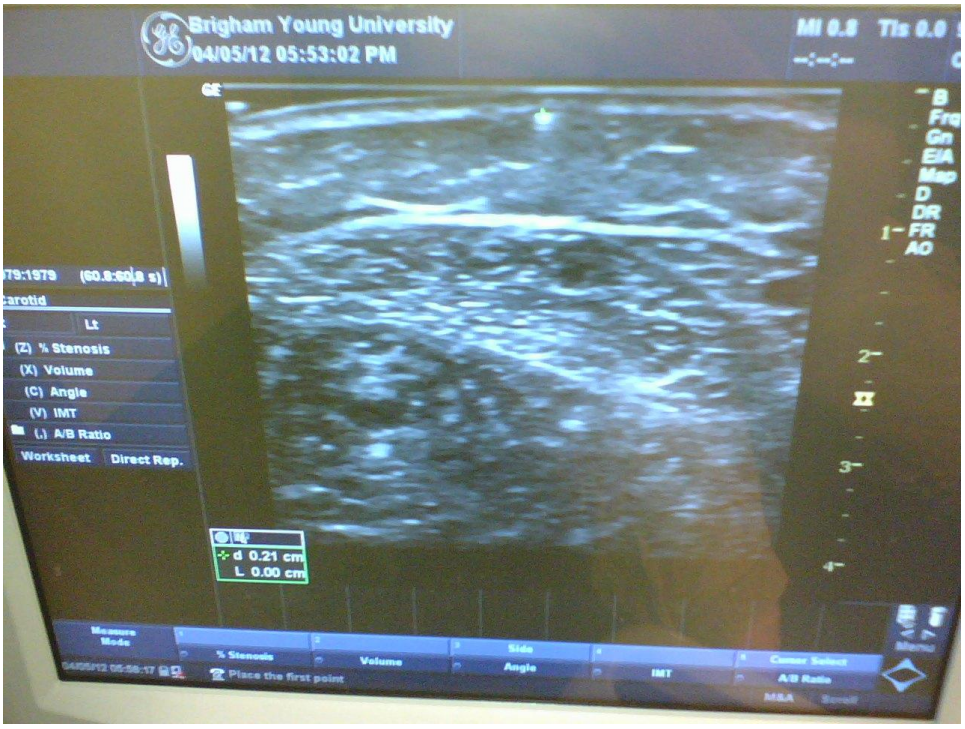

Figure 3: Phonophoresis Treatment