

Effect of Phonophoresis on Serum Salicylate Levels

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Abstract:

The purpose of this investigation was to determine the effect of ultrasound intensity and mode on serum salicylate levels following phonophoresis. Approximately 12-13 g of a salicylate product (Myoflex) was applied to the right anterior forearm of five males and two females. Randomly ordered ultrasound treatment intensities (0.0 W • cm⁻²; 1.5 W • cm⁻², pulsed 50%; and 1.5 W • cm⁻², continuous) were applied through the salicylate-containing product for a 5 min duration. A 7.0 ml blood sample was drawn from the left anterior forearm prior to each treatment and again 2 h after treatment. Analysis of variance indicated that none of the topical salicylate treatments produced an increase in serum salicylate levels. These findings suggest that there is no appreciable absorption of salicylate into the bloodstream following topical application of salicylate with or without the use of ultrasound. Since any penetration of salicylate through the skin would result in an increase in serum salicylate levels, the efficacy of phonophoresis to introduce medication into the subdermal tissue is questionable. These findings suggest that a critical review of phonophoresis in general is indicated.

Article:

STRATUM CORNEUM, MASS SPECTROMETRY

Ultrasound is frequently used in the rehabilitation of soft tissue injuries. The technique of applying ultrasound includes the use of a coupling agent to facilitate the penetration of sound waves into body tissue. Ultrasound may also be used to introduce medication into the tissue by a technique known as phonophoresis. Phonophoresis is administered in the same manner as ultrasound, except that medication is used in the coupling agent (8,18). This procedure is used to administer medication without the pain and discomfort which can accompany injections (15,17-19). Phonophoresis is usually performed with anti-inflammatory medications, such as cortisol, dexamethasone, and salicylates, and with anesthetics, such as lidocaine (1,19). It is believed to hasten rehabilitation by decreasing pain and promoting healing (1,2) and is a commonly employed technique in physical therapy clinical practice. Most studies have utilized hydrocortisone as the medication to be driven (6,7,9-12,14), although Quillen (15,16) has suggested that continuously administering a steroid to tendinous and ligamentous structures may result in their failure under stress.

The mechanism allowing medication to be driven into underlying tissues during phonophoresis is thought to be a change in tissue permeability due to the ultrasound (19). In addition, the medication could be forced away from the transducer into the body by the radiation pressure exerted by the ultrasound beam (19). The rate of diffusion could be due to acoustical streaming, which is the movement of fluids on the cell membranes due to mechanical pressure (19). Most studies have utilized the continuous mode of ultrasound (2,6,7,9-12,14,17), although the pulse mode could be used to minimize the thermal effects of ultrasound (16).

Antich (1) expressed the need for further studies involving phonophoresis with medications other than hydrocortisone. Salicylate is one such medication which has been mentioned with the use of ultrasound (1,18,19). Salicylate cream is a commonly used nonprescription topical analgesic in the form of 10% trolamine salicylate (3), a salt of salicylic acid. Salicylic acid is an active metabolite of acetylsalicylic acid. Acetylsalicylic acid, or aspirin, is a commonly used chemical administered for its analgesic and antipyretic effects and, in larger doses, for its antiinflammatory effects (4). A rapid distribution of salicylic acid occurs throughout all body tissues, with about 50-90% of it bound to plasma proteins (3). The topical application of salicylate is thought to penetrate the skin and, in combination with phonophoresis, to be driven into subdermal tissue. However, the rate of absorption has not been examined through controlled studies. Any absorption of salicylate at the subdermal level would increase serum levels. Thus, the purpose of this investigation was to determine the effect of ultrasound intensity and mode on serum salicylate levels following phonophoresis.

METHODS

Subjects

Subjects consisted of five males and two females (X age = 24 yr; X height = 177.8 cm; X weight = 75 kg) who reported no history of sensitivity to aspirin or salicylate. All subjects were healthy and did not require medication for any condition. The investigation was approved by the University of Virginia Human Investigation Committee, and all subjects gave written consent before participating in the study. Subjects were asked to refrain from taking medications which contained aspirin or salicylate compounds throughout the course of the study.

Treatments

Three phonophoresis treatments were administered using a 10% trolamine salicylate product (Myoflex, Rorer Consumer Pharmaceuticals, Fort Washington, PA) as the coupling agent. The treatments included phonophoresis with continuous ultrasound, 50% pulsed ultrasound, and ultrasound at an intensity of zero. The order of the treatments was randomized, and all treatments were performed 1 wk apart to allow clearance of salicylate from the blood.

Prior to each treatment, the left antecubital region of each subject was swabbed with alcohol, and a 7.0 ml blood sample was collected. The right anterior forearm of each subject was washed and thoroughly dried. A circle 8.0 cm in diameter was then traced onto the forearm. Approximately 12-13 g (one tablespoon) of salicylate cream was applied within the boundary of the circle. One minute following the initial application of the drug, the treatment was begun.

Following the topical application of the drug to the right anterior forearm of each subject, the ultrasound unit intensity was set at 0.0 W cm⁻²; 1.5 W cm⁻², pulsed 50%; or 1.5 W • cm⁻² continuous. The zero- intensity treatment was performed to determine whether any absorption occurred due to the topical application of the drug without the enhancement of ultrasound. The ultrasound head was used to massage the Myoflex into the tissue in the same manner as the two phonophoresis treatments.

The treatments were conducted with the use of an Intellect model 225P ultrasound unit (Chattanooga Corporation, Chattanooga, TN) with a 5 cm sound head. Calibration of the ultrasound unit was performed by the manufacturer immediately prior to the study and was confirmed immediately following completion of the treatment sessions. The treatment technique consisted of a slow circular motion with the ultrasound head, and all treatments were given by the same therapist. Treatment time was of a 5 min duration. Time was monitored on a stopwatch for greater accuracy to ensure that subjects received the same exposure to the drug. Following the treatment, the drug was immediately wiped off.

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Two hours following each of the treatments, a 7.0 ml blood sample was once again drawn from the left arm of each subject. This time duration was chosen based on previous research which indicated that average peak serum salicylic acid levels occur 2 h following an oral dose of 1,000 mg of aspirin (13). All samples were refrigerated until laboratory analysis could be completed.

Laboratory Procedures

Laboratory analysis of all blood samples was conducted at the Clinical Chemistry and Toxicology Laboratories at the University of Virginia Health Sciences Center. After clotting, blood samples were centrifuged and serum samples drawn off.

Baselt (4) suggested high-pressure liquid chromatography (HPLC) as a means to determine salicylate concentrations in the blood. However, results of a pilot study for this investigation revealed that samples from the Myoflex treatments yielded salicylate concentrations too low to be measured by HPLC. Therefore, gas chromatographic mass spectrometry was employed to measure salicylate concentrations.

A standard curve was prepared using an SMI pipet to measure 100 μ l of standard solutions of salicylic acid into screw cap culture tubes, and 0.9 ml of blank serum was added to each tube. Patient samples consisted of 1 ml of serum pipetted into the labeled screw cap culture tubes. Then 50 μ l of the internal standard containing 100 ng of tetradeuterated salicylic acid and 200 μ l of 2 N HCl were added to all the tubes. Under a hood, 5.0 ml of methylene chloride was added to every sample. Samples were then placed on a shaker (low setting) for 10 min and then centrifuged at 1,500 g for 10 min. Next, the lipid/protein layer was aspirated off the top of the methylene chloride using a vacuum. The methylene chloride extract was poured into 15 ml centrifuge tubes, and the solvent was removed under the hood using argon at room temperature.

The samples were then derivatized by adding 50 μ l of MTBSTFA, 50 μ l of acetonitrile, and 20 μ l of pyridine to each sample. Samples were then heated at 70°C for 30 min. One microliter of each sample was injected into the gas chromatograph-mass spectrometer. The mass spectrometer used was a triple quadrupole mass spectrometer (TSQ-70, Finnigen MAT, San Jose, CA) operated in the single quadrupole selected ion monitoring mode. The instrument was operated in the electron ionization mode using 70-eV electrons. The Hewlett Packard gas chromatograph was equipped with a DB-1 poly(dimethylsiloxane) bonded-phase fused silica capillary column, 5.0 m x 0.32 mm with a 0.25 μ film thickness. Serum salicylic acid (SA) concentrations were determined by monitoring peaks corresponding to the loss of C4117 \pm from the molecular ions of SA and tetradeuterated SA. These peaks for SA and tetradeuterated SA occurred at a mass to charge ratio of 309 and 313, respectively. The SA concentrations were calculated by peak height measurement relative to the tetradeuterated SA. The method was linear from 50 to 1,000 ng \cdot ml⁻¹.

Statistical Analysis

A two-within-subject-factors, repeated-measures analysis of variance was performed to examine the treatment effects. The two factors were treatment and time.

RESULTS

The means and standard deviations for the serum salicylate levels before and after the salicylate treatments and the pre- to post-treatment differences in serum salicylate levels are presented in Table 1. Analysis of variance found no significant differences for measurement time ($F(1,6) = 0.03, P > 0.05$) or the measurement-time-by-treatment interaction ($F(2,12) = 26, P > 0.05$), indicating that none of the treatments resulted in an increase in serum salicylate levels. Mass spectrometry is an extremely sensitive technique which will detect SA from exogenous and endogenous sources. Thus, pre-treatment samples read in the range of 50-100 ng \cdot ml⁻¹ as compared with 77 μ g \cdot ml⁻¹ for a patient taking 1 g of aspirin orally (4). This sensitivity allowed us to determine that less than 0.3% of the applied dose was absorbed by the treated subjects.

DISCUSSION

The results of this study indicate that there was no measurable absorption of salicylate after one topical application with or without the enhancement of ultrasound. These findings are in contrast with others (1,10,11) who demonstrated the penetration of corticosteroids into swine tissues by the use of ultrasound. Pigs were used in these studies due to the biological similarities between swine skin tissue and human skin tissue.

TABLE 1. Serum salicylate levels (ng \cdot ml⁻¹) before and after three ultrasound applications over topically applied salicylate cream (mean \pm SD).

	Ultrasound Application					
	0.0 W \cdot cm ⁻²		1.5 W \cdot cm ⁻² , pulsed 50%		1.5 W \cdot cm ⁻² , continuous	
Before	84.43	(65.73)	100.43	(128.99)	61.29	(46.45)
After	89	(60.63)	84.14	(99.12)	79.57	(48.91)
Change	+4.57	(52.58)	-15.57	(41.84)	+18.29	(47.55)

g = 10³g.

Griffin and Touchstone (11) experimented with a variety of ultrasound frequencies to determine which would be most effective for phonophoresis. The greatest concentrations of hydrocortisone were measured from the extracted pig muscle following continuous ultrasound with a frequency of 250 kc, with and without the external application of cortisol. However, this frequency produced a second degree burn on the skin of the pig. Low-intensity phonophoresis (0.3 and 0.1 W \cdot cm⁻²) was also examined by Griffin and Touchstone (10), but, because of the low intensities, long treatment times of 17 min and 51 min were indicated. These treatment durations are impractical for the clinician, and it was admitted by those authors that the cortisol may have been released by the adrenal cortex of the pig in response to the stressful situation. Cortisol has been shown to penetrate skeletal muscle (9) and paravertebral nerve (12) of pig tissues. In each case, an intensity of 1.0 W \cdot cm⁻² was used, and the continuous ultrasound was administered in a stationary manner for 5 min. The authors stated that this intensity could be tolerated by human tissues, although this is questionable. It is interesting to note that Griffin and Karselis (8) stated that a significant amount of medication can be recovered 10 cm deep to the phonophoresis treatment site after a 5 min treatment utilizing a stationary technique.

A more recent investigation by Davick et al. (6) utilized dogs for studying the effects of phonophoresis with cortisol. Two groups of dogs received ultrasound following the topical application of cortisol, with one group receiving a 10% preparation and the other group receiving a 5% preparation. The ultrasound intensity was 0.5 W \cdot cm⁻² for 8 min, utilizing a circular motion with the ultrasound head. A third group of dogs received a topical application of 10% cortisol and no ultrasound. In each case, the cortisol was wiped off 8 min after the initial application. The dogs treated with 10% cortisol and ultrasound showed significantly more penetration into the epidermis than the dogs treated with the 10% preparation alone, leading these authors to believe that the penetration of cortisol beyond the stratum corneum was due to the enhancement of ultrasound. They believed that the cortisol could easily penetrate the tissues once it was repelled by the ultrasound radiation pressure beyond the outer layer of the epidermis. Statistically, the penetration of 10% cortisol with ultrasound was slightly greater than the penetration of 5% cortisol with ultrasound.

In the study by Davick et al. (6), the animal was immediately sacrificed following the treatment. Because penetration had occurred beyond the stratum corneum, those authors believed that the drug may have begun systemic distribution and that greater amounts of cortisol may have been received if an indefinite time period had been allowed between the treatment and the sacrifice of the animal. Our investigation allowed 2 h following each of the treatments before a blood sample was collected, thus allowing time for penetration of the drug. However, the topical application of salicylate, with and without the use of ultrasound, resulted in no significant increase in serum levels. As such, it is likely that no appreciable absorption of salicylate occurred into the subdermal tissues.

The results of the present study are consistent with the findings of Benson et al. (5). In their double-blind, placebo-controlled study using human subjects, they tested the effects of pulsed and continuous ultrasound at different frequencies on the percutaneous absorption of benzydamine. Benzydamine, a nonsteroidal antiinflammatory drug, was applied to the anterior forearm of each subject 5 min prior to the ultrasound treatment in an attempt to saturate the outer layers of the skin with the drug in hopes that the ultrasound would be capable of driving the medication beyond the stratum corneum. The researchers' results led them to believe that ultrasound does not enhance the percutaneous absorption of benzydamine, although it was suggested that multiple applications over a number of days may drive in the drug which may be present in the outer layers of skin from previous treatments in which the drug was applied.

Not to be ignored, however, are clinical research studies which have supported the use of phonophoresis. Kleinkort and Wood (14) showed that phonophoresis with 10% hydrocortisone was superior to phonophoresis with 1% hydrocortisone. Of the patients treated with a 1% preparation, 79.9% were listed as improved (significant increase in range of motion and/or decrease in pain), and, of these, 33.8% were asymptomatic (no pain, full range of motion). Of the patients treated with a 10% preparation, 94.7% were improved, with 67.9% of them asymptomatic. Antich et al. (2) compared four treatments (ice, phonophoresis, iontophoresis, and ice/ultrasound contrast) for knee extensor mechanism disorders. Although it was concluded that the ice/ultrasound contrast was the superior treatment for this condition, a 32% subjective improvement was noted after only four phonophoresis treatments which utilized a preparation of 1 ml of Hexadrol and 1 ml of 4% Xylocaine.

Smith et al. (17) compared ice massage, ultrasound, iontophoresis, and phonophoresis as treatments for shin splints. Phonophoresis was applied using a mixture of 33 mg of Decadron and 16 ml of 2% lidocaine gel in 60 mg of a water-soluble base. The results indicated that none of the treatments was superior, but all were effective compared with a control group. Griffin et al. (7) performed a double-blind study in which phonophoresis was compared with ultrasound. The continuous mode was used for each group, and intensity was adjusted to patient tolerance (<1.5 W. cm²). Of the subjects who received phonophoresis with hydrocortisone, 68.1% were listed as improved (increased range of motion and decreased pain) in an average of 4.5 treatments, whereas 55.5% of the subjects receiving ultrasound with a placebo were categorized as unimproved in an average of 7.0 treatments.

The Physicians' Desk Reference for Non-Prescription Drugs (3), in describing Myoflex, states that percutaneous absorption of the drug does occur and that, following topical application in humans, blood levels have been demonstrated. However, the present study showed that one isolated application of salicylate under any of the treatment conditions failed to produce any appreciable absorption of salicylate. These results suggest that phonophoresis with salicylate preparations may not be an effective anti-inflammatory treatment. The limit of detection of systemic levels of salicylate indicated that less than 1 mg was absorbed, which would not be high enough to convince the researchers that a pharmacological effect could have taken place locally. Further study is needed using multiple applications or longer exposures of the drug to see whether indeed ultrasound can drive in any of the drug which has remained in the outer layers of skin from previous application treatments.

The results of this study and the review of the literature suggest that perhaps phonophoresis is substrate specific. In addition, we feel that a critical review of phonophoresis in general is indicated.

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