1-1-2006

Cortisol concentrations in human skeletal muscle tissue after phonophoresis with 10% hydrocortisone gel

Amanda R. Kuntz

Christine Multer Griffiths
Nova Southeastern University, cgriffit@health.usf.edu

James M. Rankin

Charles W. Armstrong

Thomas J. McLoughlin

Journal of Athletic Training 2006;41(3):321–324

www.journalofathletictraining.org

Cortisol Concentrations in Human Skeletal Muscle Tissue After Phonophoresis With 10% Hydrocortisone Gel

Amanda R. Kuntz; Christine M. Griffiths; James M. Rankin; Charles W. Armstrong; Thomas J. McLoughlin

The University of Toledo, Toledo, OH

Amanda R. Kuntz, MS, ATC, contributed to conception and design; acquisition and analysis and interpretation of the data; and drafting, critical revision, and final approval of the article. Christine M. Griffiths, PhD, ATC, contributed to conception and design; acquisition of the data; and critical revision and final approval of the article. James M. Rankin, PhD, ATC, and Charles W. Armstrong, PhD, contributed to conception and design and critical revision and final approval of the article. Thomas J. McLoughlin, PhD, ATC, contributed to acquisition and analysis and interpretation of the data and drafting, critical revision, and final approval of the article.

Address correspondence to Thomas J. McLoughlin, PhD, ATC, The University of Toledo, Department of Kinesiology, 2801 West Bancroft Street, Toledo, OH 43606. Address e-mail to thomas.mcloughlin@utoledo.edu.

Context: The delivery of hydrocortisone through phonophoresis is a widely prescribed technique for the treatment of various musculoskeletal inflammatory conditions. However, limited scientific evidence exists to support the efficacy of phonophoresis in delivering hydrocortisone to skeletal muscle tissue in humans.

Objective: To determine hydrocortisone (cortisol) concentrations in human skeletal muscle tissue after a phonophoresis treatment using 10% hydrocortisone gel.

Design: Randomized design in which 12 subjects were randomly assigned to either an ultrasound (sham) treatment or a 10% hydrocortisone phonophoresis treatment.

Setting: Laboratory.

Patients or Other Participants: Twelve healthy subjects (8 women, 4 men: age = 22.3 ± 2.64 years, height = 168.28 ± 8.19 cm, mass = 69.58 ± 9.05 kg) with no history of musculoskeletal disease, preexisting inflammatory conditions, or recent orthopaedic injuries.

Intervention(s): Ultrasound at 1.0 MHz, 1.0 W/cm^2, at a continuous setting for 7 minutes was applied to a standardized area of the vastus lateralis muscle in both groups. The contralateral limb served as the control (no treatment) for both the sham and the phonophoresis groups.

Main Outcome Measure(s): Vastus lateralis muscle biopsies were taken from both legs immediately after treatment, and cortisol concentrations were analyzed using an enzyme-linked immunosorbent assay.

Results: We observed no significant difference in muscle cortisol concentration between the contralateral control limb and the treatment limb in either the sham or the phonophoresis group ($P > .05$). No significant difference was noted when the treatment limbs in the sham and phonophoresis groups were compared ($P > .05$).

Conclusions: Our data suggest that a 10% hydrocortisone-based phonophoresis treatment did not increase cortisol concentrations in human skeletal muscle tissue.

Key Words: ultrasound, ELISA, inflammation, biopsy, hydrocortisone

Phonophoresis, a technique in which ultrasound is used to increase the transcutaneous transmission of drugs, is a widely applied clinical technique for the treatment of musculoskeletal inflammation. Specifically, phonophoretic delivery of hydrocortisone, a synthetic equivalent of the endogenously produced anti-inflammatory corticosteroid cortisol, is purported to provide relief of symptoms associated with arthritis, tendinitis, and bursitis.[1–3] Although a few authors using animal models (eg, porcine, canine) have provided disparate results regarding the ability of phonophoresis to deliver hydrocortisone to musculoskeletal tissue,[4–6] no scientific evidence is currently available demonstrating the ability of phonophoresis to deliver hydrocortisone to subcutaneous tissue in humans. The paucity of literature regarding the efficacy of phonophoresis in the delivery of drugs to subcutaneous tissues precludes any definitive conclusions regarding its potential usefulness in the management of inflammation in humans.

Therefore, our purpose was to examine the efficacy of phonophoresis in the delivery of hydrocortisone to skeletal muscle tissue in humans using commonly accepted clinical values. A human model was chosen because (1) extraction of skeletal muscle tissue from human subjects is a viable approach, and (2) phonophoretic delivery of hydrocortisone is a promising avenue for the treatment of skeletal muscle inflammation associated with a variety of muscle traumas in humans (eg, strains, crush induced, thermal insults). Considering the demonstrated transdermal drug delivery potential of ultrasound,[7] we hypothesized that an increase in skeletal muscle tissue cortisol concentrations would result after a standard hydrocortisone-based phonophoresis treatment.

METHODS

Subjects

Twelve apparently healthy subjects (8 women, 4 men: age = 22.3 ± 2.64 years, height = 168.28 ± 8.19 cm, mass = 69.58 ± 9.05 kg) with no history of musculoskeletal disease, preexisting inflammatory conditions, or recent orthopaedic injuries participated in the study. All subjects provided written informed consent before participating. Procedures pertinent to the study were performed in accordance with guidelines established by The University of Toledo Human Subjects Research and Review Committee.

Experimental Design

In a randomized design, each subject was assigned to either an ultrasound (sham) (n = 6; 4 women, 2 men) or phonophoresis (n = 6; 4 women, 2 men) treatment group. After the subjects were allocated to treatment groups, 1 leg was randomly allocated to receive either sham or phonophoresis treatment. The other leg served as the contralateral control (untreated) in all subjects. To control for potential diurnal influences, all subjects reported to the laboratory between 11:00 AM and 12:00 PM. Subjects were instructed not to participate in exercise or any excess physical activity before reporting to the laboratory.

Treatment Settings

All ultrasound treatments (sham and phonophoresis) were administered by the same researcher (an athletic trainer) using an Omnisound 3000B machine (Accelerated Care Plus, Reno, NV) with a constant transducer head speed of approximately 2.0 cm/s. A 5-cm^2 sound head was used with the values at 1 MHz, 1.0 W/cm^2, at a continuous setting. The treatment was applied for 7 minutes directly over the vastus lateralis muscle, using a 10-cm^2 template to ensure that treatment remained in the designated area. These ultrasound settings were selected according to previous findings[8–10] and are in agreement with standard accepted clinical practice for phonophoresis administration. These treatment values were selected to capture both the thermal and nonthermal effects of ultrasound in order to optimize transdermal hydrocortisone delivery.[11]

For the sham condition, ultrasound was administered using 5 mL of Aquasonic 100 ultrasound transmission gel (Parker Laboratories, Inc, Fairfield, NJ). For the phonophoresis treatment, ultrasound was administered using 5 mL of Aquasonic ultrasound transmission gel that was supplemented with 10% hydrocortisone (prepared by an Ohio-licensed compounding pharmacist). This hydrocortisone concentration was chosen to coincide with the purported benefit of 10% hydrocortisone-based phonophoresis treatment[2] and is in accordance with current standard clinical practice. Any excess gel was wiped away upon completion of the treatment.

Muscle Biopsy

Before the treatment was initiated, an area of skin over the vastus lateralis muscles bilaterally was shaved in preparation for ultrasound administration and muscle biopsy. Immediately after ultrasound treatment, the area was sanitized using an antiseptic solution, and a small area over the belly of the vastus lateralis muscle was then anesthetized via a subcutaneous injection of 2 mL of 1% lidocaine. After 10 minutes, a muscle biopsy, using the protocol established by Bergstrom,[12] was taken from both legs. Briefly, a sterile biopsy needle (5 mm) was inserted through an incision of approximately 1 cm and a muscle sample (approximately 100 mg) extracted from the belly of the vastus lateralis.[13] The order of the biopsy procedure (treated versus untreated leg) was randomly determined. Muscle samples were snap frozen in isopentane precooled with dry ice and immediately stored at −80°C for further processing.

Cortisol Analysis

Muscle tissue samples were homogenized in phosphate-buffered saline (pH = 7.4) and cortisol extracted according to manufacturer's guidelines (Oxford Biomedical Research Inc, Oxford, MI). Briefly, muscle homogenates were diluted 1:10 in ethyl ether and vortexed to facilitate phase separation. The organic phase was extracted and evaporated under a stream of nitrogen gas. The precipitate was then dissolved in 100 μL of diluted extraction buffer (Oxford Biomedical) and cortisol measured via an enzyme-linked immunosorbent assay (ELISA; Oxford Biomedical). All samples were analyzed in duplicate via spectroscopy (SpectraMax 190, 650 nm; Molecular Devices, Sunnyvale, CA) and average concentrations used for statistical analyses. Samples are reported in ng/mg muscle tissue.

Statistical Analysis

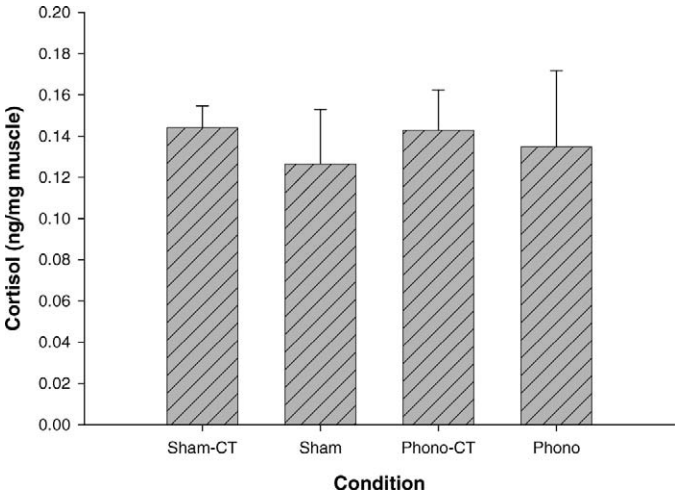
We used a paired-samples *t* test to analyze baseline muscle cortisol concentrations between the contralateral control limb and treatment limb for both the sham (ultrasound) and phonophoresis groups. After baseline analysis was performed, we calculated an independent *t* test to analyze muscle cortisol concentrations between the respective treatment limbs for both the sham and phonophoresis groups. Significance was determined at $P \leq .05$ for all statistical procedures.

RESULTS

We observed no significant difference ($P = .38$) in muscle cortisol concentrations between the contralateral control limb and respective treatment limb in either the sham (mean ± SEM: 0.1442 ± 0.0106 ng/mg muscle) or phonophoresis groups (0.1428 ± 0.0196 ng/mg muscle) (Figure). No significant difference ($P = .51$) in muscle cortisol concentrations was noted when we compared the treatment limbs in the sham (0.1263 ± 0.0266 ng/mg muscle) and phonophoresis groups (0.1348 ± 0.0368 ng/mg muscle) (see Figure). All cortisol levels fell within the accepted reference range for the ELISA (0.04–10 ng/mL; Oxford Biomedical).

DISCUSSION

Phonophoresis has been shown to be effective for driving low-molecular-weight compounds (eg, insulin: molecular mass = 6000 d) through the dermal layer,[7,14] thus providing a promising, noninvasive approach for the delivery of a myriad of medications (eg, hydrocortisone: molecular weight = 342 d) to subcutaneous tissues. Once through the skin, however, the ability of phonophoresis to provide targeted delivery of drugs to specific tissues is uncertain. To our knowledge, this is the first study to address the effectiveness of phono-



Effect of 10% hydrocortisone-based phonophoresis on skeletal muscle tissue cortisol concentrations. The contralateral limb served as the control (CT) for both the ultrasound (sham) and phonophoresis (phono) groups. Data represent mean ± SEM.

phoresis in the targeted delivery of anti-inflammatory medication to skeletal muscle tissue in humans. In this study, hydrocortisone (cortisol) concentrations in skeletal muscle tissue after 10% hydrocortisone-based phonophoresis treatment were not significantly elevated over concentrations after sham treatment. Although previous authors[4–6,15] using various animal models have reported disparate findings on muscle cortisol concentrations after hydrocortisone-based phonophoresis, our findings indicate that standard phonophoresis treatment, using the defined settings, was ineffective for the delivery of hydrocortisone to skeletal muscle tissue in humans.

Controversy exists within the literature regarding the effectiveness of ultrasound in the delivery of cortisol into muscle tissue. In agreement with our findings, Davick et al[6] observed no increase in muscle cortisol concentrations in canines after phonophoresis treatment using a tritiated cortisol cream. Although Davick et al indicated that the cortisol was able to penetrate the skin, their findings did not demonstrate elevated cortisol concentrations within the underlying skeletal muscle tissue. We did not assess hydrocortisone penetration, but it is conceivable that the administration of ultrasound resulted in the penetration of cortisol into the subdermal layers surrounding the muscle tissue.[16] Therefore, it is possible that the phonophoresis treatment did not result in an increase in muscle cortisol concentrations but could have been absorbed in the extracellular environment of the muscle and redistributed through uptake by the surrounding vasculature. However, Bare et al[15] observed no significant difference in serum cortisol concentrations after a 10% hydrocortisone phonophoresis treatment similar to the settings we used, indicating that the drug did not diffuse into the circulation. As a result, it is unlikely that the phonophoresis treatment we used resulted in an increase in blood cortisol concentrations.

In contrast, previous authors have demonstrated an increase in intramuscular cortisol concentrations after phonophoresis treatment. In landmark studies, Griffin and Touchstone[4,5] observed an increase in cortisol in skeletal muscle tissue after both ultrasound and phonophoresis treatments in swine using ultrasound intensities of 0.1, 0.3, 1.0, and 3.0 W/cm^2. Generally, the observed increases in cortisol were greatest with prolonged phonophoresis treatments (ie, 17–51 minutes) using the lower ultrasound intensities of 0.1 and 0.3 W/cm^2, indicating that skin permeability may be enhanced with lower-intensity, longer-duration ultrasound treatments.[5] Provided that these observations transcend to the human model, it is possible that a protracted treatment of low-intensity phonophoresis may be required to drive hydrocortisone into skeletal muscle tissue in humans. Investigation into divergent ultrasound treatment settings on muscle tissue cortisol concentrations after hydrocortisone-based phonophoresis in humans may be warranted.

Although it is evident from the present findings that hydrocortisone-based phonophoresis using the settings defined within this study did not result in an increase in intramuscular cortisol concentrations in humans, uninjured tissue may be resistant to exogenous pharmaceutical delivery. For instance, Cagnie et al[9] found ketoprofen (a nonsteroidal anti-inflammatory drug) concentrations to be higher in synovial tissue after ketoprofen-based phonophoresis treatment in patients with knee injuries. We reasoned that hydrocortisone, being a lipophilic molecule that can readily traverse the lipid bilayer of cell membranes,[17] should be able to access the sarcoplasm of intact skeletal muscle cells. However, injured muscle has been shown to demonstrate an up-regulation in glucocorticoid receptor availability[18] and an increase in cellular endocytosis,[19,20] which may make the muscle cells more amenable to the effects of hydrocortisone-based phonophoresis.

Although it cannot be determined in the present study, factors such as inadequate skin hydration[21] and/or suboptimal heating of either the stratum corneum or subcutaneous tissues might have hindered ultrasound-mediated transdermal drug transport. The thermal influence of ultrasound on the permeability of the dermal layer has received some support,[22] but Mitragotri et al[7] demonstrated that the nonthermal and mechanical effects of therapeutic ultrasound (ie, frequency range = 1–3 MHz, intensity range = 0–2 W/cm^2) appear to play a more significant role in transdermal drug delivery. Although the ultrasound settings we used likely provided both thermal and nonthermal effects at the cutaneous and subcutaneous levels,[11] the degree to which these ultrasound effects need to be attained for targeted subcutaneous drug transmission in humans is a potential avenue of future research.

In conclusion, a diverse array of treatment strategies has been developed to help ameliorate the purported negative side effects associated with skeletal muscle injury and inflammation. Hydrocortisone-based phonophoresis is a widely prescribed therapeutic modality that may provide transient relief of discomfort associated with various musculoskeletal maladies.[1,3] In light of the present findings, however, the value of a standard hydrocortisone-based phonophoresis treatment for the management of skeletal muscle inflammation appears to be debatable. More expansive studies (eg, larger sample sizes) examining the efficacy of different ultrasound settings for drug delivery to damaged muscle and associated tissue (eg, tendon, bursa) may be warranted to detect any increases in skeletal muscle cortisol elicited after phonophoresis treatment.

ACKNOWLEDGMENTS

We thank Aaron Schreiner for assisting with data collection, Drs Francis X. Pizza and Jeffrey Barley for technical contributions, Dr Phillip A. Gribble for critical insight into the manuscript preparation, and Rudolph Medical Equipment & Supply Co, Inc (West Chester, OH), for providing the Omnisound 3000B ultrasound unit.

REFERENCES

1. Griffin JE, Echternach JL, Price RE, Touchstone JC. Patients treated with ultrasonic driven hydrocortisone and with ultrasound alone. *Phys Ther.* 1967;47:594–601.
2. Kleinkort JA, Wood F. Phonophoresis with 1 percent versus 10 percent hydrocortisone. *Phys Ther.* 1975;55:1320–1324.
3. Nehra R, Achadinha T, Patel T. Ultrasound relief improves with hydrocortisone. *Biomechanics.* 2003;11:77–83.
4. Griffin JE, Touchstone JC. Ultrasonic movement of cortisol into pig tissues, I: movement into skeletal muscle. *Am J Phys Med.* 1963;42:77–85.
5. Griffin JE, Touchstone JC. Low-intensity phonophoresis of cortisol in swine. *Phys Ther.* 1968;48:1336–1344.
6. Davick JP, Martin RK, Albright JP. Distribution and deposition of tritiated cortisol using phonophoresis. *Phys Ther.* 1988;68:1672–1675.
7. Mitragotri S, Edwards DA, Blankschtein D, Langer R. A mechanistic study of ultrasonically-enhanced transdermal drug delivery. *J Pharm Sci.* 1995;84:697–706.
8. Klaiman MD, Shrader JA, Danoff JV, Hicks JE, Pesce WJ, Ferland J. Phonophoresis versus ultrasound in the treatment of common musculoskeletal conditions. *Med Sci Sports Exerc.* 1998;30:1349–1355.
9. Cagnie B, Vinck E, Rimbaut S, Vanderstraeten G. Phonophoresis versus topical application of ketoprofen: comparison between tissue and plasma levels. *Phys Ther.* 2003;83:707–712.
10. Leonard J, Merrick M, Ingersoll C, Cordova ML. A comparison of intramuscular temperatures during 10-minute 1.0-MHz ultrasound treatments at different intensities. *J Sport Rehabil.* 2004;13:244–254.
11. Byl NN. The use of ultrasound as an enhancer for transcutaneous drug delivery: phonophoresis. *Phys Ther.* 1995;75:539–553.
12. Bergstrom J. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. *Scand J Clin Lab Invest.* 1975;35:609–616.
13. Evans WJ, Phinney SD, Young VR. Suction applied to a muscle biopsy maximizes sample size. *Med Sci Sports Exerc.* 1982;14:101–102.
14. Mitragotri S, Blankschtein D, Langer R. Ultrasound-mediated transdermal protein delivery. *Science.* 1995;269:850–853.
15. Bare AC, McAnaw MB, Pritchard AE, et al. Phonophoretic delivery of 10% hydrocortisone through the epidermis of humans as determined by serum cortisol concentrations. *Phys Ther.* 1996;76:738–749.
16. Ueda H, Sugibayashi K, Morimoto Y. Skin penetration-enhancing effect of drugs by phonophoresis. *J Control Release.* 1995;37:291–297.
17. Guyton A, Hall J. *Textbook of Medical Physiology.* 10th ed. Philadelphia, PA: WB Saunders; 2000.
18. Willoughby DS, Taylor M, Taylor L. Glucocorticoid receptor and ubiquitin expression after repeated eccentric exercise. *Med Sci Sports Exerc.* 2003;35:2023–2031.
19. Eddleman CS, Ballinger ML, Smyers ME, Godell CM, Fishman HM, Bittner GD. Repair of plasmalemmal lesions by vesicles. *Proc Natl Acad Sci U S A.* 1997;94:4745–4750.
20. Pizza FX, McLoughlin TJ, McGregor SJ, Calomeni EP, Gunning WT. Neutrophils injure cultured skeletal myotubes. *Am J Physiol Cell Physiol.* 2001;281:C335–C341.
21. Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. *Eur J Pharm Sci.* 2001;14:101–114.
22. Bommannan D, Menon GK, Okuyama H, Elias PM, Guy RH. Sonophoresis, II: examination of the mechanism(s) of ultrasound-enhanced transdermal drug delivery. *Pharm Res.* 1992;9:1043–1047.