Continuous Therapeutic Ultrasound Accelerates Repair of Contraction-Induced Skeletal Muscle Damage in Rats

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Objective: To examine the effect of ultrasonography on the repair of muscle injured through repeated eccentric contractions.

Design: Randomized, case-control study.

Setting: Laboratory animal facility.

Animals: Thirty-three Sprague-Dawley rats.

Interventions: Specimens were anesthetized and each foot strapped, in turn, onto a motorized foot pedal, which moved the ankle repeatedly through a range of 110°. Extensor digitorum longus muscles were injured by stimulating the peroneal nerve during foot plantarflexion. Continuous 1.0MHz ultrasound treatments at 0.5W/cm² were applied through water submersion technique to 1 limb daily for 3, 5, or 7 days postinjury. The contralateral limb served as an injured control.

Main Outcome Measures: Extensor digitorum longus maximum isometric tetanic force (Pₒ) was measured in vitro from all extensor digitorum longus muscles and used as a functional index of muscle injury.

Results: Analysis of variance and Tukey post hoc analysis showed no significant differences in Pₒ between treated and untreated limbs at 3 and 5 days, but at 7 days postinjury, Pₒ of ultrasound-treated muscle was significantly higher than was untreated muscle.

Conclusion: Seven days of continuous therapeutic ultrasound improved force production after contraction-induced muscle injury.

Key Words: Exercise; Foot injuries; Muscle, skeletal; Rats, Sprague-Dawley; Rehabilitation; Ultrasonography.

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ULTRASONOGRAPHY, a physical modality that uses high-frequency sound waves to penetrate to deeply situated soft tissue, helps augment the body’s natural healing process for tendon injuries and in scar formation. Much is also known of ultrasound’s effect on blood flow. However, there is little information about its effect on skeletal muscle injury in general, and specifically, its effect on the repair of muscle that has been damaged through repeated eccentric (or lengthening) contractions. Eccentric contractions cause more muscle injury, as measured by histologic analysis or decrements in maximum isometric tetanic force (Pₒ), than shortening or isometric contractions. Muscle injury that is associated with eccentric-based activity is common in sports and recreation and is also becoming prevalent among aged individuals who are active physically. Aged persons are more susceptible to exercise-induced skeletal muscle injury, and have a decreased capacity for repair. Any intervention that accelerates the healing process, such as ultrasound, would be particularly beneficial to these individuals.

This study investigated the effect of continuous therapeutic ultrasound on the repair of skeletal muscle injured through repeated eccentric contractions.

METHODS

Thirty-nine male Sprague-Dawley rats (weight, 112–391g) were used in this study. Prior approval was obtained from our Institutional Laboratory Animal Care and Use Committee.

Animals were assigned to 1 of 5 groups in which extensor digitorum longus muscles were injured bilaterally through repeated eccentric contractions. Continuous 1.0MHz ultrasound treatments at 0.5W/cm² were applied to 1 lower limb daily for 3 days (n = 7 rats), 5 days (n = 8), or 7 days (n = 7) postinjury. The contralateral limb served as injured control. The ultrasound intensity of 0.5W/cm² was used to minimize its thermal effect. A separate group of animals (n = 7) served as noninjured controls; both limbs of these animals were uninjured and did not receive ultrasound treatment. A fifth group of animals (n = 4) served as an injured-control group; their limbs were injured but did not receive ultrasound treatment.

Injury Protocol

The rats were anesthetized with xylazine (7mg/kg body weight [BW] intraperitoneally [IP]) and ketamine HCl (60mg/kg BW IP); incisions were made on the lateral surface of both knees, and the common peroneal nerves were isolated. Stainless steel electrodes were placed under each nerve. The animal was then placed on a flexion-extension injury device, and each hindlimb was injured separately. Each foot was secured to a motorized footplate with surgical tape. The footplate repeatedly flexed and extended the ankle through a range of 110° at a frequency of 21 cycles/min. Electric stimulation was applied to the peroneal nerve during ankle plantarflexion, while the extensor digitorum longus muscle was forcibly lengthened. Muscles were stimulated with an SD5 stimulator that delivered pulses of 5 volts for 0.2ms and at a frequency of 150Hz. Contractions were repeated in two 5-minute bouts with a 5-minute rest between each bout. This protocol produces a measurable and consistent damage to the extensor digitorum longus muscle that closely simulates contraction-induced injury in humans. Immediately after the injury procedure, animals were removed from the apparatus, incisions were su-
tured, and Betadine solution applied to the incision sites. Continuous therapeutic ultrasound was then applied to randomly selected limbs in the animals that were randomly assigned to treatment groups. The extensor digitorum longus muscle from animals in the noninjured control group were not injured. Animals assigned to the noninjured control and injured control groups were given a short rest before the muscle was removed and force production measured. The rest minimized the likelihood that short-term fatigue would contribute to changes in force.

Because active muscle strain affects the degree of exercise-induced injury, we calculated the changes in fiber lengths under each condition from measured changes in total muscle length during the flexion-extension protocol. Fiber length (L_f) was calculated by multiplying the muscle’s optimum muscle length (L_o) by a L_f/L_o ratio of .399.18

Ultrasound Treatment

Animals randomly assigned to ultrasound treatment groups received 5-minute applications of continuous 1.0MHz ultrasound2 at 0.5W/cm² to either the left or right hindlimb. Ultrasound treatments were started immediately after injury and continued daily for 3, 5, or 7 days. At the same time each day, animals were removed from their cage, sedated with Domitor (medetomidine hydrochloride, 40µg/kg subcutaneously), and suspended in a cloth harness over a tub of degassed water. The hindlimb to be treated was lowered into the water and the extensor digitorum longus muscle positioned within 3mm of, and perpendicular to, a stationary 5-cm transducer. The other hindlimb did not receive ultrasound treatment and served as an injured, control limb. After ultrasound treatment, animals received a subcutaneous injection of Antisedan (atipamezole hydrochloride, 1.0µg/kg) to reverse the effects of Domitor.

Measurement of Maximum Tetanic Force

P_o is a reliable, functional index of muscle injury16,18-20 and was measured in vitro using a dual-chamber tissue bath.2 The outer bath was filled with 25°C water21 and the inner tissue reservoir filled with buffer solution containing in mM 1:1

- 154 NaCl, 33 NaHCO₃, 6 KCl, 3 CaCl₂, 1.5 MgSO₄, 1.5 KH₂PO₄, and 1.0g/L glucose. A mixture of 95% oxygen to 5% carbon dioxide was bubbled continuously through the solution to ensure sufficient oxygenation of the tissue while maintaining the pH at 7.35 to 7.45.

To measure P_o of extensor digitorum longus muscles, animals were anesthetized as described, and each muscle was isolated and removed from the hindlimbs separately. The proximal tendon was attached to an adjustable clamp in the tissue bath while the distal tendon was glued to a plastic arm (0.5×8.6cm) that was screwed onto the lever arm of a dual-mode servomotor.4 The muscle was allowed to stabilize for approximately 20 minutes in the tissue bath.21 Muscles were electrically stimulated with platinum plate electrodes (0.5×2.0cm). Force output was displayed on, and stored in, a 2-channel storage oscilloscope.5 The servomotor was calibrated daily with lead weights.

Optimal voltage was established by measuring twitch force, then gradually increasing the voltage until no corresponding increase in force was recorded. Optimum length (L_o) was determined by gradually increasing the muscle length until no increase in twitch force was noted. After optimal voltage and optimal length were determined, a force/frequency curve was established for each muscle by stimulating at frequencies of 20 to 180Hz, in increments of 20Hz. Muscles were stimulated for 500ms at each frequency and were allowed to recover for 2 minutes between contractions. Maximum tetanic force was set at the level when no further increase in force was observed with an increase in stimulation frequency. Muscles were then removed from the bath, trimmed of visible fat and connective tissue, and weighed on a Mettler balance. Physiologic cross-sectional area (PCSA) for the extensor digitorum longus muscle was calculated22 as:

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\text{Muscle mass (g)} = \text{Fiber length (cm)} \times \text{muscle density (g/cm}^2)\]

A muscle density of 1.056g/cm² was used22 and L_f was calculated for each muscle as previously described.21 P_o was normalized by expressing force per PCSA in N/cm².

Statistical Analysis

A one-way analysis of variance (ANOVA) (P < .05) was used to compare extensor digitorum longus muscle force data from all limbs, and Tukey honestly significant difference (HSD) post hoc comparison was used to test for statistical differences in force between groups.

RESULTS

One-way ANOVA revealed significant differences in P_o between groups (F 7 = 6.848, P < .000). Results of Tukey HSD post hoc analysis showed P_o of injured extensor digitorum longus muscles from both treated and untreated groups to be significantly lower than P_o of the control uninjured group at 3 and 5 days postinjury. By 7 days postinjury, however, P_o of treated muscles was significantly higher than untreated muscle and was not different from control uninjured muscle (fig 1).

DISCUSSION

Results of this study indicate that 7 daily treatments of continuous therapeutic ultrasound significantly improves maximum force production of muscles that have been injured by repeated eccentric contraction, compared with injured but untreated controls. Results from previous studies of the effectiveness of ultrasound in aiding tissue repair are contradictory. Some investigators found no treatment effect on regenerating skeletal myofibers, fibroblasts, or in accelerating repair of ulcers and wounds. Others have reported positive results, observing a treatment effect on tendon injuries, wound healing, and lateral epicondylitis. Reasons for these different
findings are not readily apparent, perhaps because the mechanisms through which ultrasound affects tissue repair are obscure. Whether a continuous or a pulsed mode of ultrasound is used does not appear to be the variable that determines treatment effect, for both application modes have been both successful and unsuccessful in showing treatment effects in different studies. A combination of factors, including the type of tissue examined, model of injury, and mode, intensity, and frequency of ultrasound treatment, may account for the different findings. There seems little doubt, however, that ultrasound treatment accelerates cell proliferation, at least for fibroblasts and satellite cells.

The mechanism through which ultrasound helps tissue repair is likely related to its mechanical effects (ultrasonication), as opposed to the thermal effect. Ultrasonication, or “micromassage” of the tissue, produces a change in membrane permeability and stimulates the transport of second messenger substances, such as calcium, across the cell membrane. These second messengers may then stimulate the proliferation of myogenic cells—in the case of skeletal muscle—the satellite cells. Satellite cells are presumptive myoblasts located between the basal lamina and cell membrane of skeletal muscle fibers. They are normally quiescent, but as a result of muscle damage are activated and reenter the replicative phase of the cell cycle. Satellite cells can form completely new fibers in the event of cell death or can help repair a focal injury that does not destroy the whole fiber. The “state” of a satellite cell largely relies on the interplay among 3 growth factors: fibroblast growth factor (FGF), insulin-like growth factor I (IGF-I), and transforming growth factor B (TGF-B). Cell injury stimulates the release of a mitogenic factor, which activates satellite cells. In the early stages of regeneration, FGF and IGF-I are present in all 3 growth factors, which inhibits differentiation, stimulates cell proliferation. It is in this stage that therapeutic ultrasound may have its greatest effect. Rantanen et al observed an increase in satellite cell number in regenerating skeletal muscle after pulsed ultrasound treatment, compared with injured untreated muscle. Others noted an increase in the cell proliferative stage of tissue repair as a result of ultrasound treatment.

The next stage of the repair process, cell fusion and differentiation, is regulated by IGF-I in the absence of FGF and TGF-B. In their model of muscle injury, Rantanen observed no increase in the number of myotubes in this second stage of repair, suggesting no real benefit of ultrasound treatment although satellite cell proliferation had been stimulated by ultrasound in the first stage. However, they did not perform any studies on the regenerating muscle. In our study, we noticed an increase in function by 7 days postinjury, suggesting that ultrasound stimulation of satellite cell proliferation was followed by an increased rate of differentiation.

The difference between our findings and those of Rantanen’s may center on 2 factors: the model of injury and the parameters of applied ultrasonography. Rantanen used a contusion injury, which resulted in a transverse rupture of the muscle belly. Our injury was produced by forcibly lengthening the muscle while it was stimulated to contract. This contraction-induced injury typically produces an initial mechanical injury limited to the area of the 2-line, followed by a secondary injury that may be related to oxygen-free radical production associated with inflammatory processes. In our model, even if the cell is completely destroyed, the basal lamina provides a scaffolding on which the regenerating fiber can rebuild. This condition was not present in the contusion model used by Rantanen, in which fibers are ruptured. Another difference between the 2 studies was the parameters of ultrasound treatment. We treated for 5 minutes daily with continuous 1.0MHz at 05.0W/cm², whereas Rantanen treated injured muscles for 6 minutes using pulsed (20% duty cycle) 3.0MHz ultrasound at 1.5W/cm². Furthermore, although we started ultrasound treatment immediately after injury, Rantanen waited 3 days before initiating treatments and used a 2 days on, 1 day off treatment regimen.

It is unclear whether the differences between the 2 studies account for their different findings. Ultrasound may stimulate satellite cell proliferation in the early stages of regeneration, but whether it affects differentiation, by influencing the interplay among the 3 regulatory growth factors mentioned earlier, is not certain. Ultrasound may have an effect on the rate of synthesis and/or release of 1 or all 3 growth factors, or satellite cell sensitivity to 1 or all may be altered. The mode, intensity, and frequency of application of ultrasound may also affect its effectiveness.

**CONCLUSION**

Although the mechanisms through which ultrasonography affect tissue repair remain obscure, our results support a beneficial effect of immediate application of 7 daily treatments of continuous ultrasound in the accelerated repair of skeletal muscle injured by repeated eccentric contractions.

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Suppliers

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c. Harvard Apparatus Inc, 84 October Hill Rd, Holliston, MA 01746.
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