A Comparison of Continuous Ultrasound and Pulsed Ultrasound on Soft Tissue Injury Markers in the Rat

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Abstract. The cellular effects of pulsed and continuous ultrasound on the protein content of muscle after trauma were determined in this study. Rats were subjected to a single impact trauma to the gastrocnemius. Six major treatment groups were employed: (1) uninjured control, (2) injured control, (3) & (4) uninjured ultrasound treated, and (5) & (6) injured ultrasound treated. The ultrasound treated groups were subdivided into continuous ultrasound, and pulsed ultrasound treatments (dosage = 1.0 watts/cm² × 5 min), which were given from day 2 to day 7 post-trauma once daily. Animals were sacrificed at 1, 3 and 7 days, the medial gastrocnemius muscle being dissected and solubilized for protein content determination. On day 7 the control injured group had a statistically greater mean protein content than the injured continuous ultrasound treatment group, and the mean protein content of the injured pulsed ultrasound treatment group was statistically greater than both the injured control or the injured continuous ultrasound treatment groups (one way ANOVA, P<0.05). The means of all three uninjured control groups were not significantly different at day 1 or 7. Within each group, only the control injured (18% or 44 mg increase ± 17 mg) and the injured pulsed ultrasound treatment (7% or 22 mg increase ± 9 mg) groups showed statistically significant changes (95% Confidence Interval) in protein content from day 1 to day 7. All other treatment groups showed no statistically significant increases.

Key words: Ultrasound, Continuous, Pulsed, Soft tissue, Rat

INTRODUCTION

Therapeutic ultrasound is a modality commonly used by physiotherapists for the treatment of soft tissue injuries (Maxwell 1992). Various authors have suggested that the biophysical effects resulting from the interaction of ultrasound with tissue can be grouped into the classifications of thermal (continuous) and non-thermal (pulsed) effects (Dyson 1978). The thermal effects are produced by attenuation of the sound wave as it passes through the tissue. This process of attenuation is caused by the conversion of ultrasonic energy into heat. The non-thermal effects of ultrasound involve stable acoustic streaming and cavitation. It is further reported that non-thermal effects can best be achieved by controlling the intensity of the ultrasonic energy (Prentice 1999, Michlovitz 1996).

A number of studies have reported the thermal phenomena of therapeutic ultrasound on increased tissue temperature (Draper et al. 1993, 1995). The majority of these thermal effects occur at tissue temperatures between 40 to 45 degrees centigrade. Williams (1987) found that when ultrasound was
applied at 1 MHz with an intensity of 1 W/cm², tissue temperature increased at a rate of 0.86 degrees centigrade per minute. Prentice (1999) and Dyson (1987) have shown that increases in tissue temperature will occur with both pulsed or continuous ultrasound treatments but the increase in temperature is dependent on the intensity of the current.

The thermal effects of ultrasound are reported to be most beneficial when increases in tissue temperature are desired. This deep heating treatment is reported to increase collagen tissue extensibility, alter blood flow, enhance enzymatic activity within tissues, and increase the contraction time of the muscle (Dyson 1987, Michlovitz 1996).

The non-thermal effects of ultrasound include cavitation and acoustic micro streaming. Stable cavitation is the vibrational effect of gas bubbles within the tissue when the ultrasound beam comes into contact with them. Acoustic micro streaming is the unidirectional movement of fluid along the wall or membrane of the cell resulting from the mechanical pressure of the ultrasonic wave (Prentice 1999). Dyson (1978) found that the therapeutic benefits of non-thermal ultrasound were derived only by stable cavitation, which maintains the integrity of the gas-filled bubbles within the ultra structure or micro streaming which does not compromise the membrane of the cell.

The non-thermal effects of ultrasound on injured soft tissue may be more effective than the thermal effects because the mechanical pressure of the wave produces changes in the movement of ions across the cell membrane, which in turn may aid in the recovery of the damaged cell (Maxwell 1992). A number of reports have non-thermal ultrasound to stimulate protein synthesis, increase blood flow and enhance bone healing, all of which aid in the recovery process (Dyson and Luke 1986, Hogan et al. 1982, Pilla et al. 1990).

When treating a soft tissue injury the goal of the therapist is to decrease the recovery time and return the injured tissues to the original state (Reid 1992). Much of the information directly relating to how ultrasound effects soft tissue is based on macroscopic observations (Stratton et al. 1984, Maxwell 1992), and few studies focus directly on the cellular response of the injured tissue following the application of ultrasound (Stratton et al. 1984). One study suggested that ultrasound enhances protein synthesis and cell division (Ramirez et al. 1997). Others reported that ultrasound stimulated the rate of growth of replacement tissue at the site of pressure sores (Dyson and Suckling 1978). Ultrasound has also been reported to reduce inflammation by directly inducing mast cells to release histamine, which in turn produces vasodilation and increased vascular permeability (Fyfe and Chahl 1984). In a separate experiment, the same authors also injected silver nitrate into the rat’s hind paw and examined the effects of various ultrasound frequencies on plasma extravasation. They reported that ultrasound decreased plasma extravasation (Fyfe and Chahl 1980). Cortisol, which is known for its strong anti-inflammatory effects, was also found to increase in plasma concentrations following the application of ultrasound. Griffin (1966) reported that following sonication over nerve plexes and peripheral nerves, the production of cortisol increased resulting in decreased pain and inflammation.

**MATERIALS AND METHODS**

**Animals**

All procedures described below were carried out in conformance with the principles for the care and use of animals of the American Physiological Society, and the Canadian Council on Animal Care. The rats, weighing 200 gm–250 gm at the start of the experiment, were housed individually in wire cages and provided with commercial rat chow (Wayne Lab Blox) and water ad libitum. The one hundred and eight male Sprague-Dawley rats were maintained on a 12-h light: 12-h dark cycle.

Rats were subjected to a single impact trauma to the medial aspect of the gastrocnemius of the right leg, using an approved injury device (Fisher et al. 1989). During this procedure rats were briefly anaesthetized with halothane. The medial side of the calf muscle was padded with a layer of gauze (0.5 cm depth) to prevent tearing of the skin. The trauma was delivered by dropping a solid aluminum cylinder with a flat impact surface (1.38 cm × 27 cm in length, weighting 700 gm) once only through a distance of 125 mm, onto the padded muscle. The instantaneous force delivered by a falling object with these characteristics was calculated to equal 0.57 Newton-meters/cm², where a Newton-meter is equal to the force of an object weighing 100 gm falling over a distance of 1 meter. Since the surface area of the impact device was 1.5
cm², the force delivered was 0.57 newton-meters/cm². To ensure stabilization and accurate delivery, the cylinder was dropped down a tubular guide fixed to a ring stand. The limb was positioned manually with the foot stabilized at a 90-degree angle to the tibia. The location of the belly of the gastrocnemius was determined by palpation and the device placed over its broadest region. The tibia was protected from accidental fracture by carefully placing it out of the line of travel of the device; manual placement of the limb was employed primarily to avoid bone fracture.

Four major treatment groups were employed:
I. Uninjured control (no treatment)
II. Injured control (no treatment)
III. Uninjured ultrasound treated (control)
IV. Injured ultrasound treated

Animals were sacrificed at 1, 3 and 7 days post-trauma. The medial gastrocnemius muscle was dissected whole at the time of sacrifice. Tissues were solubilized in 1.0 M NaOH at room temperature and protein was determined according to the Bradford methods using bovine serum albumin as standard (Bradford 1976). Results are expressed in protein mass per muscle. All data are expressed as group means with the standard deviation or standard error of the mean. Statistical comparisons were done using unpaired t-tests and one way ANOVA, and p<0.05 was considered significant.

RESULTS
The effects of pulsed and continuous ultrasound on the protein content of the medial gastrocnemius muscle following acute blunt trauma were determined. On day 7 the mean protein content of the control injured group (μ = 291 mg ± 5 mg, SE 2 mg) was statistically (one way ANOVA P<0.01) greater than the mean protein content of the injured continuous ultrasound treatment group (μ = 264 mg ± 11 mg, SE 5 mg). The mean protein content of the injured pulsed ultrasound treatment group was 341 mg ± 6 mg (SE = 3 mg), which was statistically greater than both the injured control and injured continuous ultrasound treatment groups (one way ANOVA p<0.01). The means of all three uninjured control groups were not significantly different from one another at day 1 or 7.

When examining a one way analysis of variance (ANOVA) for the protein content change within each treatment group from day 1 to day 7, only the control injured group and the injured pulsed ultrasound treatment group showed statistically significant changes (95% Confidence Interval) in mean protein content. The injured control group showed a 44 mg increase (± 17 mg) in mean protein content (18%) and the injured pulsed ultrasound treatment group showed a 22 mg (± 9 mg) mean increase (7%) both of which were significant increases (one way ANOVA P<0.01). All other treatment groups showed no statistically significant signs of increases in protein content. The injured
continuous ultrasound treatment group showed a mean increase in protein content of 4% or 10.5 mg (± 19.18 mg), which was not statistically significant. All uninjured treatment groups showed no significant signs of protein content change.

When comparing the amount of change between groups, the control injured treatment group showed a statistically significant greater increase in protein content compared to the pulsed ultrasound injured group (one way ANOVA P<0.05). The continuous ultrasound injured treatment group was significantly below the average change of the injured control group (one way ANOVA p<0.05) but not significantly below the pulsed ultrasound injured treatment group (one way ANOVA P<0.05). Likewise, the change in protein content seen in the continuous and pulsed ultrasound injured treatment groups did not show statistically significant differences, the pulsed ultrasound having the higher amount of change.

### DISCUSSION

In response to the injury, the muscles that received the pulsed ultrasound responded in a therapeutic manner with a significant production of contractile protein. Following an acute injury, there is an inflammatory response, which continues for three days. Inflammation is important because it allows inflammatory cells to enter the injury site and remove the damaged tissue. In additional studies we have demonstrated that this removal of injured tissue is due to macrophage-associated lysosomal proteolysis and occurs within the first forty-eight hours following trauma. Following this time period, the muscle is free to regenerate the production of new proteins. Studies using similar techniques as ours have shown that this increase in muscle protein content is related to the satellite cell production of skeletal myofibrils, and to promote myoregeneration rather than scarring, the availability of myogenic cells could be increased with the application of pulsed ultrasound.

The muscle injury protocol used in this study is an experimental model of muscle contusion of moderate severity. During the first 3 days after acute blunt trauma there was a marked reduction in protein mass of the injured muscle at the site of injuries in our previous studies. Protein repletion occurred over the next 4 days in the untreated, injured limb; and this process was complete by day 7 when the treated muscle with pulsed ultrasound was compared to the controlled non-injured muscle. We tested ultrasound therapy during the protein repletion phase after muscle trauma. The efficacy of a treatment may be evaluated by a variety of criteria, such as histology (Stratten 1984), the concentration of cellular (Fyfe and Chahl, 1984) prostaglandins (Fisher et al. 1994) and the regeneration of skeletal muscle following injury (Rantanen et al. 1999). Here we have used overall replacement of tissue protein with a fixed time period as a measure of healing rate in treated and untreated animals. Measures of tissue protein mass used here are not susceptible to artifact resulting from changes in tissue water content (oedema). Using this criterion, therapeutic ultrasound has heterogeneous effects on protein mass of muscles.

In the medial gastrocnemius muscle of the injured limb, there was an increase in tissue protein mass with both the continuous and pulsed ultrasound treatments as well as with the injured control. This treatment thus appeared to promote regenerative events leading to protein repletion in

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Day 1 mean (mg) (± SD (mg))</th>
<th>Day 7 mean (mg) (± SD (mg))</th>
<th>Change of the mean (mg) (± SD (mg))</th>
<th>%Change of the mean (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Uninjured rats</td>
<td>342 (42)</td>
<td>360 (32)</td>
<td>+18 (55)</td>
<td>5 (16)</td>
</tr>
<tr>
<td>Control Injured rats</td>
<td>248 (14)</td>
<td>291 (5)</td>
<td>+44 (17)†</td>
<td>18 (7)†</td>
</tr>
<tr>
<td>Continuous Ultrasound treated, Uninjured rats</td>
<td>331 (33)</td>
<td>341 (16)</td>
<td>+10 (30)</td>
<td>3 (10)</td>
</tr>
<tr>
<td>Continuous Ultrasound treated, Injured rats</td>
<td>254 (22)</td>
<td>264 (11)</td>
<td>+11 (19)</td>
<td>4 (7)</td>
</tr>
<tr>
<td>Pulsed Ultrasound treated, Uninjured rats</td>
<td>364 (15)</td>
<td>361 (15)</td>
<td>–3 (21)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Pulsed ultrasound treated, Injured rats</td>
<td>319 (4)</td>
<td>341 (6)</td>
<td>+22 (9)†</td>
<td>7 (3)†</td>
</tr>
</tbody>
</table>

†: Statistically significant change (one way ANOVA p<0.05, 95% Confidence Interval).
the treated muscles, and the animals injured and treated with the pulsed ultrasound showed the greatest therapeutic effect when compared to their counterparts - injured and treated with continuous ultrasound. Ultrasound therefore appears to induce a positive anabolic effect on muscle when it is treated with pulsed ultrasound. Present data do not suggest a mechanism for this treatment effect.

Stratton et al. (1984) studied the effects of ultrasound following acute blunt trauma to muscles. Rats were subjected to a single blow to the hind limb and then were treated with ultrasound, sacrificed and the traumatized muscle was examined by light microscopy. Counts of muscle nuclei were used as an indicator of regeneration; a dosage of 1.5 watts per square centimeter was highly significant as compared to a low dosage of 0.05 watts per square centimeter in promoting muscle regeneration. Fisher et al. have shown that when ultrasound is delivered at 1.5 watts per square centimeter, prostaglandin production is down-regulated in injured muscle tissue which offsets protein degradation and aids in the recovery of the injured muscle. Rantanen et al. (1999), using a similar method to Fisher (1989), produced muscle injury in rats and reported that pulsed ultrasound was more effective in stimulating satellite cell production. The beneficial effects on satellite cells were due to ultrasound’s high frequency vibration energy at the site of the injured tissue and this in turn leads to a stimulatory micromassaging effect of myoblasts to donate or produce satellite cells.

Much of the information directly relating to the effects of ultrasound on soft tissue lesions is based on macroscopic observations (Dyson and Suckling 1978, Prentice 1999), and very few studies have focused directly on the cellular response of injured tissue following the application of therapeutic ultrasound (Rantanen et al. 1999). In addition most of these studies have been on non-muscle tissue (Maxwell 1992, Rantanen et al. 1999). Early studies by Harvey et al. (1975) suggest that ultrasound enhances protein synthesis in fibroblast. Ultrasound has also been reported to reduce inflammation by directly inducing mast cells to release histamine, which in turn causes vasodilatation and increased vascular permeability (Fyfe and Chahl 1984, Prentice 1999). Several investigators have reported that ultrasound enhances tissue healing. Dyson and Suckling (1978) reported in their early studies that low dosages of pulsed ultrasound increased tissue growth and regeneration in injured rabbit’s ear. Rantanen et al. (1999) helps muscle to recover following injury by stimulating satellite cell proliferation and Mantone et al. (2000) found ultrasound promoted muscle healing in the injured shoulder of athletes.

Ultrasound continues to be a frequently used modality for the treatment of soft tissue injury (Prentice 1999, Fisher 1994, Rantanen 1999). Despite the frequent clinical use of this modality, many of the physiological effects of ultrasound are still not well understood (Montone et al. 2000). The presence of a positive effect of ultrasound reported in this study support the use of pulsed ultrasound in the treatment of acute muscle injury.

REFERENCES


