THERAPEUTIC ULTRASOUND AND IMMOBILIZATION IN MUSCULAR TRAUMA REPAIR

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SUMMARY

Introduction: We assessed the effects of therapeutic ultrasound (TUS), either added to cast immobilization (CI) as a treatment alternative to muscular injuries caused by impact by assessing the mechanical properties of stretching and load at proportionality and maximum limit, stiffness (S) and gastrocnemius muscle resiliency. Methods: 70 female rats were employed in the study, and the animals were divided into 7 groups: Group 1- Control; Group 2- Untreated; Group 3- CI for 24 hours; Group 4- CI for 72 hours; Group 5- TUS without CI; Group 6- CI for 24 hours combined with TUS; Group 7- CI for 72 hours combined with TUS. Results: Loads at proportionality limit and maximum limit showed that the group receiving TUS behaved similarly to control group. The property of stretching at proportionality limit was not different from one group to another; the maximum stretching of the group receiving TUS and of the groups immobilized for 72 hours was comparable to control group. Conclusion: The group receiving TUS showed similar stiffness levels compared to control group and superior resiliency compared to all remaining groups. The standalone use of TUS provided similar results to those regarded as normal, but these were not noticed when TUS was combined to CI.

Keywords: *Muscle injury. Immobilization. Therapeutic ultrasound. Biomechanics.*

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INTRODUCTION

Currently, different therapeutic alternatives are being suggested targeting the full recovery of patients with muscle injuries within the shortest possible time, thus providing them back with a normal functional physical status and allowing a better physical performance.[1]

TUS has been used for over six decades to treat soft tissues, being currently a resource strongly employed in physiotherapeutic practice[2], and the most used one for treating soft tissues' injuries.[3]

The effects of the TUS on muscular repair process in experimental injuries have been studied under different aspects.

Stratton, Heckmann and Francis[4] used different therapeutic ultrasound powers for the histochemical evaluation of its effects for the repair process of blunt muscle injuries, regarding it as beneficial.

Rantanen et al.[5] concluded that therapeutic ultrasound accelerates muscle repair after contusion promoting significant proliferation of satellite cells to the injury site.

Menezes et al.[6] applied therapeutic ultrasound in an experimental muscle injury model by smashing, acquired their results by means of mechanical assays and concluded that it seems to have an improvement of the injury repair quality. Injured muscles have not been previously treated with immobilization.

Karnes and Burton[7] found a significant improvement of the muscle strength degree, in an injury caused by repeated eccentric contraction, when the injury was stimulated with therapeutic ultrasound.

Järvinen[8] and Järvinen et al.[9] showed the benefits of early immobilization as a part of the treatment of an injured muscle. Järvinen et al.[10] recommended rest as a prompt treatment approach in muscle injuries, accompanied by local ice, compression and injured limb lifting, this method being widely employed in daily clinical practice.

We didn't find in literature studies correlating the effects of therapeutic ultrasound with plastered immobilization on muscle repair and the corresponding mechanical properties.

Our objective was to assess the influence of TUS, added by plastered immobilization or not, after immediate trauma, on the process of muscle repair by assessing the mechanical properties of muscular fibers of gastrocnemius muscle.

MATERIALS AND METHODS

Experimental animals

Seventy female albino Wistar rats weighting 204 ± 15g and 10-12 weeks old were used. The animals were kept in separate plastic restrain cages, with water and food ad libitum, being exposed to bright/ dark environments of 12-h each until the experimental injury was produced.

All experimental procedures in the study complied with the rules and ethical principles for animal experimentation, as approved by the Committee of Ethics in Animal Experimentation (CEUA – University of São Paulo, Ribeirão Preto campus).

The animals were divided into 7 experimental groups according to the treatment protocol to be adopted.

All the authors state no potential conflict of interest concerning this article.

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Group 1 - Control (N=10)

The animals included in this group were not submitted to any injury, remaining in restraint cages for a period of 7 days.

Group 2 – Untreated (N=10)

These animals had their gastrocnemius muscle submitted to acute experimental injury by mechanism of impact; however, following trauma, no therapeutic resource was applied, being kept in their restraint cages for 7 days and free active mobilization.

Group 3 – Immobilized for 24 hours (N=10)

After the acute experimental injury was produced, this group of animals was immobilized for 24 hours by means of plastered device including hip, knee and ankle joints of the right limb. When the period was completed, plastered immobilization was removed, and the animals were kept in their restraint cages for additional 6 days.

Group 4 – Immobilized for 72 hours (N=10)

In this experimental group, the animals also had their gastrocnemius muscle submitted to injury production, being immobilized according to the same protocol as described for group 3, but for a 72-hour period. When this period was completed, the plastered immobilization was removed and the animals were kept in their restraint cages for additional 4 days.

Group 5 – Stimulation with TUS (N=10)

After the muscle injury was produced, the animals remained in their restraint cages for 24 hours, and then stimulated with pulsed therapeutic ultrasound (TUS) for 5 minutes for 6 consecutive days.

Group 6 – Immobilized for 24 hours and stimulation with TUS (N=10)

The animals in this group were submitted to muscle injury production process, being immediately submitted to immobilization with plastered device. After that period, the plastered device was removed and the animals were stimulated with pulsed therapeutic ultrasound (TUS) for 5 minutes for 6 consecutive days.

Group 7 – Immobilized for 72 hours and stimulation with TUS (N=10)

The animals were submitted to the experimental injury production, being immediately immobilized. After 24 hours of immobilization, stimulation on the injured area was initiated, the access to which was achieved by a window produced on the plaster cast, with pulsed therapeutic ultrasound (TUS) for 5 minutes for 6 consecutive days.

Experimental contusion

A tool able to produce a muscle injury by mechanism of impact caused by a 200g load release 30 cm over the gastrocnemius muscle, with the animals properly positioned on a metal surface on tool's base. This tool was developed at the Bioengineering Laboratory of the University of São Paulo, the same as the one used by Oliveira et al.[11] consisting of an adaptation of the models described by Stratton et al.[4] and Minamoto et al.[12]

All animals were previously anesthetized with Thiopental® - sodium Thiopental– at a dosage of 4 mg/100g – administered intraperitoneally. The animals were manually immobilized, being positioned in pronation, with the thigh-femur joint extended and directly touching the metal surface, taking care to keep the maximum knee extension and dorsiflexion at 90° from the ankle (Figure 1 – A and B).

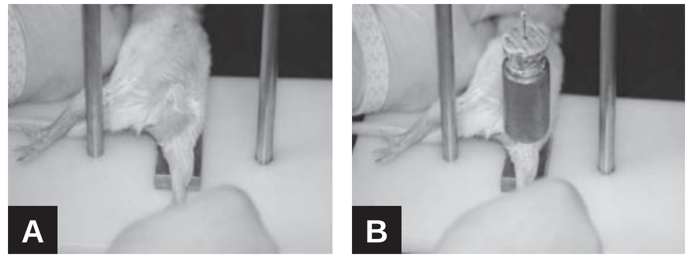

Figure 1 – *(A) Positioning of the gastrocnemius muscle for producing an experimental injury. (B) Simulation of the impact load at the final position over the gastrocnemius muscle.*

The animals were submitted to a single trauma and immediately separated according to their experimental groups.

Plastered immobilization

After the experimental injury was produced, the animals from experimental groups 3, 4, 6 and 7, while still anesthetized, were immobilized with a plastered device made of fast-dry plaster bandage, applied in a conventional way.

The plastered immobilization model adopted in this study was based on the method suggested by Booth and Kelso.[13] Such immobilization included the torso, going through the hip and knee at full extension until ankle joint, with was positioned at plantar flexion.

On the animals from group 7, a 16-mm wide rounded window was made on the plastered device over the injured muscle area in order to allow ultrasound to be applied.

Ultrasound therapy started 24 hours after muscle injury and the removal of the plastered immobilization occurred as established by the protocol on the different experimental groups.

The animals were submitted to therapeutic ultrasound sessions on a pulsed rate and modulated frequency of 100Hz, with a duty cycle of 1:5 (2ms ON and 8ms OFF – 20%), 1 MHz frequency and 0.5 W/cm^2 (Spatial Average Temporal Average) on a daily basis, for 6 consecutive days, for 5 minutes a day, always applied at the same time of the day.

Ultrasound was applied directly on the affected muscle area, by means of a 1.5 cm^2 ERA headstock, using water-soluble gel to remove the air between the interfaces.

Gastrocnemius muscle preparation

After 7 days, the animals in each experimental group were sacrificed by cardio-respiratory arrest following the administration of excessive anesthetics dosages. The right lower limb was removed by disarticulation of the hip. Tibia and the other soft parts of the right leg were removed by taking the necessary care to keep only the gastrocnemius muscle and its bone insertions at the distal femur and calcaneus, avoiding additional injuries. Thus, the specimens were created and submitted to mechanical assays.

Mechanical assays

The mechanical properties of the specimens were identified by longitudinal traction assays at a Universal Assay Machine owned by the Bioengineering Laboratory at USP Medical School, Ribeirão Preto campus.

A 50kgf load cell was employed, which presents a direct interface with a PC with mechanical assay automation software, allowing for accurate comparisons of loads and stretching achieved at each mechanical assay.

A 200g pre-load was applied with an accommodation time of 30 seconds and load application speed determined as 10mm/minute. (Figure 2 - A and B)

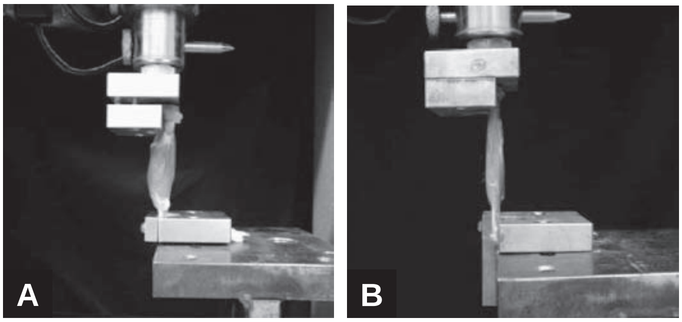

Figure 2 – *(A) Positioning of the collected specimen, together with the devices employed for fixation at the universal assay machine. (B) Simulation of the mechanical assay of longitudinal traction of the collected gastrocnemius muscle.*

Graphs for load versus stretching were built from the results of each assay, enabling the determination of mechanical properties of load and stretching at the proportionality limit, load and stretching at maximum limit, stiffness and resiliency for each specimen. (Figure 3)

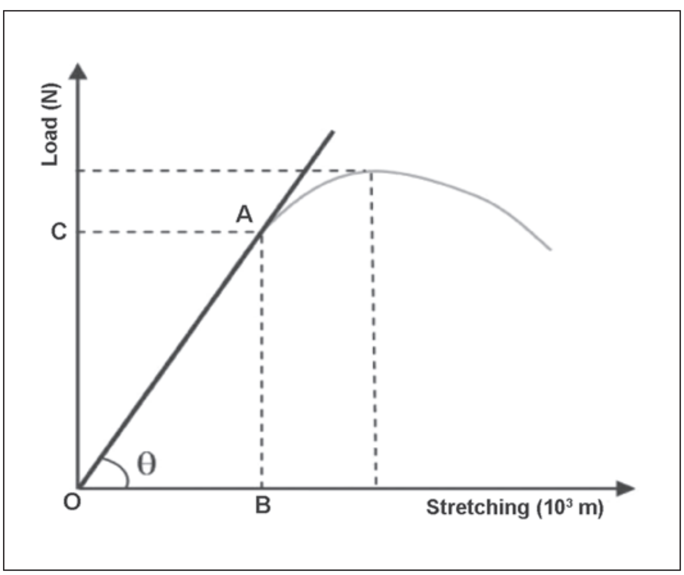

Figure 3 – *Load versus stretching curve obtained on the mechanical assay, where the assessed mechanical properties are determined.*

The data achieved on the assays were assessed by the variance analysis test – ANOVA, and by the Student-Newman-Keuls'test for comparison between groups, both with significance levels established as 5%, and processed by the Instat Graphpad® software, v.3.00.

RESULTS

The results found for each specimen were summed and the arithmetic mean values and standard deviations were calculated by using a Microsoft® Excel® 2000 application for each experimental group.

The data were processed by means of the Instat Graph-Pad® software v.3.00 to provide a statistical analysis of the results found between the different groups.

Stretching at proportionality limit

The mean values found for stretching at the proportionality limit for the control group were (4.48 ± 0.88) $x10^{-3}$ m; for group 2, (6.36 ± 2.28) $x10^{-3}$ m; for group 3, (4.78 ± 1.22) $x10^{-3}$ m; group 4, (4.91±1.46) $x10^{-3}$ m; group 5, (5.60 ± 0.65) $x10^{-3}$ m; group 6, (5.14 ± 1.72) $x10^{-3}$ m, and; group 7, (4.73±1.10) $x10^{-3}$m. No statistically significant differences were found on the simultaneous analysis of the experimental groups ($p>0.05$).

Load at proportionality limit

The mean values found for load at proportionality limit for the control group were (17.77 ± 2.10) N; group 2, (13.50 ± 3,80) N; group 3, (13.86 ± 2.66) N; group 4, (14.21 ± 5.03) N; group 5, (19.80 ± 3.60) N; group 6, (14.10 ± 2.80) N, and; group 7, (14.08 ± 3.05) N. The results found in the assays showed a statistically significant difference in the simultaneous analysis of experimental groups, with $p<0.0001$. No statistically significant difference was found for the comparison of groups 1 and 6. In the comparisons between groups 1 and 5 with the other experimental groups, statistically significant differences were found.

Maximum stretching

The mean values found for maximum stretching on the control group were (11.66 ± 2.23) $x10^{-3}$ m; group 2, (8.91 ± 2.04) $x10^{-3}$ m; group 3, (8.83 ± 1.04) $x10^{-3}$ m; group 4, (10.43 ± 1.45) $x10^{-3}$ m; group 5, (10.44 ± 1.58) $x10^{-3}$ m; group 6, (9.82 ± 3.21) $x10^{-3}$ m, and; group 7, (16.66 ± 1.49) $x10^{-3}$m. The comparison of the results found for this property showed a statistically significant difference, with $p<0.0001$. No statistically significant differences were found when the groups were compared to each other, except for groups 7.

Maximum load

The mean values found for maximum load on the control group were (31.6 ± 2,7) N; group 2, (17.7 ± 3.7) N, group 3, (22.0 ± 2.9) N; group 4, (21.5 ± 3.1) N; group 5, (28.7 ± 2.7) N; group 6, (18.2 ± 5.0) N, and; group 7, (22.5 ± 2.3) N. In the statistical analysis of the mean values obtained for maximum load, a statistically significant difference was found in the simultaneous analysis of the experimental groups, with $p<0.0001$. No statistically significant difference was found when comparing groups 1 and 5 or between groups 2,3,4,6 and 7.

Stiffness

The mean value found for stiffness on the control group was (4.047 ± 0.707) $x10^3$ N/m; on group 2, (2.239 ± 0.584) $x10^3$ N/m; group 3, (2.990 ± 0.547) $x10^3$ N/m; group 4, (2.808 ± 0.306) $x10^3$ N/m; group 5, (3.658 ± 0.676) $x10^3$ N/m; group 6, (2.860 ± 0.503) $x10^3$ N/m, and; group 7, (3.205 ± 0.492) $x10^3$ N/m. The stiffness comparison evidenced the presence of a statistically significant difference for the simultaneous analysis, with $p<0.0001$. No statistically significant differences were found in the comparison between groups 1 and 5 and on the other comparisons between groups 2, 3, 4, 6 and 7.

Resiliency

The mean value found for resiliency on the control group was (40.3 ± 11.7) $x10^{-3}$ J; on group 2, (37.1 ± 16.8) $x10^{-3}$ J; group 3, (34.2 ± 14.4) $x10^{-3}$ J; group 4, (37.9 ± 24.4) $x10^{-3}$ J; group 5, (57.2 ± 13.5)

$\times 10^{-3}$ J; group 6, $(37.9 \pm 19.0) \times 10^{-3}$ J, and; group 7, $(36.8 \pm 15.36) \times 10^{-3}$ J. In the resiliency comparison, a statistically significant difference was found on the simultaneous analysis of the experimental groups, with $p<0.05$. Statistically significant differences were found on the comparisons between group 5 and the other groups.

DISCUSSION

Mechanical properties measurement consists of a very useful tool, because it provides relevant knowledge about the resulting adaptations and changes of different functional demands.[14]

Because mechanical assays are destructive and due to the challenges of appropriately measuring the cross-sectional area at the site of muscle injury made us to decide for assessing data by means of the load versus stretching curve instead of the tension versus deformation.

We must consider that muscle injury repair is done by cells forming new muscle fibers or by cells forming fibrous tissue at the injury site. We didn't find in literature any mention concerning the behavior of a repaired muscle, correlating the value achieved by the mechanical tests and the kind of repair tissue. We don't know if the recovery of the ability to withstand load by an injured muscle is correlated to a fibrous cicatricial repair, or if this mechanical recovery is related to a better biological recovery of the muscle. Experimentally, it was demonstrated that after the first day of trauma, muscles tested in tension showed rupture on their intact portion, suggesting that the regenerated tissue acquired stronger resistance than the ruptured muscle tissue.[4,15,16]

Our mechanical assay was conducted on the 7th day, while Menezes et al.[6] carried out their assays on the 13th post-injury day. We also present different results from these authors' concerning the injured muscle and the kind of injury, once they have used smashed anterior thigh rectus muscles while we have produced a direct trauma on the calf, which caused an experimental injury of the gastrocnemius muscle.

The advantage of using gastrocnemius muscle comes from the fact that we can isolate it with its bone insertions, enabling it to be safely fixated on the test machine, avoiding the frequent loosening when the muscle is directly fixated to the clasps.

Our results were also different from those reported by Menezes et al.[6] for the speed of application on the traction assay, ours being 10 mm/minute and theirs, 4.5 mm/minute.

The property of load at proportionality limit showed favorable results to the use of therapeutic ultrasound both in our study and in the one conducted by Menezes et al.[6] However, we had favorable results to the use of ultrasound also for maximum load, which was not found on their study.

Also concerning stretching, our results are controversial, once our tests did not differentiate our groups regarding stretching at the proportionality limit, as did Menezes et al group.[6] The maximum stretching did not differentiate the groups from these authors, but, in our study, the group submitted to ultrasound stimulation and also the one immobilized for 72 hours provided comparable results between these groups and the control group. The results found in our study are similar to those reported by Menezes, et al.[6] for resiliency (energy absorbed at the elastic phase) and different for stiffness.

For muscle rehabilitation and repair process, stiffness is essential, because muscles with less stiffness stretch more when lighter loads are present.[10]

It is difficult to justify the discrepancy of results, which can be attributed to the fact that we have used different muscles, different ways to fixate on the assay machine and different follow-up periods after the injury. However, in our study, there is a trend of the results to favor the groups treated with ultrasound, and the same trend is noticed on the study by Menezes et al.[6], although not in the same mechanical properties assessed.

We expected that early immobilization after trauma, for a short period, could favor the results of mechanical resistance recovery for these muscles when associated to therapeutic ultrasound, but this did not occur.

CONCLUSION

The standalone use of TUS provided similar results to those regarded as normal on assays for load at proportionality limit and maximum load. The association of TUS with plastered immobilization for 72 hours provided comparable results to control group only for the maximum stretching property. Our results suggest that the combination of immobilization and ultrasound as a adjuvant treatment does not bring benefits for muscle repair by mechanical assays. These results achieved in laboratory animals should not be directly extrapolated to clinical practice, serving as a primary basis for further research.

REFERENCES

1. Lopes AS Kattan R, Costa S, Moura CE, Lopes RS. Diagnóstico e tratamento das contusões musculares. Rev Bras Ortop. 1995;30:744-52.
2. Warden SJ, Mcmeeken JM. Ultrasound usage and dosage in sports physiotherapy. Ultrasound Med Biol. 2002;28:1075-80.
3. Young SR, Dyson M. The effect of therapeutic ultrasound on angiogenesis. Ultrasound Med Biol. 1990;16:261-9.
4. Stratton SA, Heckmann R, Francis RS. Therapeutic ultrasound, its effects on the integrity of a nonpenetrating wound. J Orthop Sports Phys Ther. 1984;5:278-81.
5. Rantanen J, Thorsson O, Wollmer P, Hurme T, Kalimo H. Effects of therapeutic ultrasound on the regeneration of skeletal myofibers after experimental muscle injury. Am J Sports Med. 1999; 27:54-9.
6. Menezes D.F, Volpon JB, Shimano AC. Aplicação do ultra-som terapêutico em lesão muscular experimental aguda. Rev Bras Fisioter. 1999;4:27-31.
7. Karnes JL, Burton HW. Continuous therapeutic ultrasound accelerates repair of contraction induced skeletal muscle damage in rats. Arch Phys Med Rehabil. 2002; 83:1-4.
8. Järvinen MJ. Immobilization effect on the tensile properties of striated muscle: an experimental study in the rat. Arch Phys Med Rehabil. 1977; 58:123-7.
9. Järvinen MJ, Aho AJ, Lehto M, Toivonen H. Age dependent repair of muscle rupture. A histological and microangiographical study in rats. Acta Orthop Scand. 1983;54:64-74.
10. Järvinen MJ, Einola SA, Virtanen EO. Effect of the position of immobilization upon the tensile properties of the rat gastrocnemius muscle. Arch Phys Med Rehabil. 1992;73:253-7.
11. Oliveira FB, Abrahao GS, Carvalho LC, Shimano AC, Volpon JB, Picado CHF. Os efeitos do ultra-som terapêutico auxiliado a imobilização gessada nas propriedades mecânicas da reparação muscular. Estudo experimental em ratas. Rev Hosp Clin Fac Med de Ribeirão Preto USP. 2003;36:23-4.
12. Mimamoto VB, Grazziano CR, Salvini TF. Effect of single and periodical contusion on the rat soleus muscle at different stages of regeneration. Anat Rec. 1999;254:281-7.
13. Booth FW, Kelso JR. Production of rat muscle atrophy by cast fixation. J Appl Physiol. 1973;34:404-6.
14. Caiozzo VJ. Plasticity of skeletal muscle phenotype: mechanical consequences. Muscle Nerve. 2002;26:740-68.
15. Crisco JJ, Jokl P, Heinen GT, Connell MD, Panjabi MM. A muscle contusion injury model. Biomechanics, physiology, and histology. Am J Sports Med. 1994;22:702-10.
16. Järvinen MJ. Healing of a crush injury in rat striated muscle. Part.2. A histological study of the effect of early mobilization and immobilization on the repair processes. Acta Pathol Microbiol Scand Am. 1975;83:269-82.