Comparison of continuous and pulsed ultrasound therapy in knee hyperalgesia of Wistar rats

Comparação do ultrassom terapêutico contínuo e pulsado na hiperalgésia de joelho de ratos Wistar

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INTRODUCTION

Among electrothermal agents used in physiotherapy, ultrasound is one of the most common therapies and equipment is available and frequent in the clinical setting. However, in spite of its intensive use, review studies have shown that there is not enough evidence to support beneficial ultrasound effects with current parameters used in the clinical practice. Anti-inflammatory effects are credited to this resource, which afterward would be responsible for analgesia, due to increased local temperature. However, important non-thermal effects...
have been currently presented, which would be mediated by cell sodium and calcium concentrations, which would produce direct analgesic actions by changing cell depolarization and activation thresholds, even being able to be the stimulation basis for local endogenous opioids release. Studies have contradictory results of therapeutic ultrasound in different types of injuries, having among studied variables pain, such as impingement syndromes, ankle torsions, low back pain and knee osteoarthritis. However, the diversity of both clinical and research parameters is extreme, thus being useful controlled experiments to test different ultrasound treatment parameters, such as different doses, frequencies and working cycles, as well as continuous and pulsed ultrasound, which have presented different results, even when the same mean temporal and spatial intensity (SATA) is used.

So, this study aimed at evaluating and comparing the effects of therapeutic ultrasound in different presentations on hyperalgesia and edema induced by 5% formalin injection in the knees of Wistar rats.

**METHODS**

Eighteen Wistar rats, weighing 436.0±33.0g, were kept in polypropylene cages with free access to water and food, with controlled 12h light/dark cycle and controlled room temperature (24±1º C). Animals were randomly divided in three groups:

- **CG** – animals submitted to right knee hyperesthesia induction and not treated;
- **GUP** – animals submitted to right knee hyperesthesia induction and treated with pulsed therapeutic ultrasound 5:5;
- **GUC** – animals submitted to right knee hyperesthesia induction and treated with continuous therapeutic ultrasound. Animals were manually restrained and 100µL of 5% formalin were injected in the right tibio-femoral joint space, aiming at inducing synovitis, with hyperesthesia and edema.

**Nociception evaluation**

Insight Von Frey digital filament was used to evaluate nociception. Test was carried out with animals manually restrained and filament applied to the medial face of right hind paw tibio-femoral joint. Filament polypropylene tip was applied perpendicularly to the area, with gradual pressure increase and test was interrupted at clinching for clinching threshold recording. Before test, animals were trained with the equipment, for three days, aiming at their familiarization. The day after the last training, clinching threshold values were collected in moments pre-injury (EV1), 15 (EV2), 30 (EV3) and 60 (EV4) minutes after chemical irritation.

**Edema evaluation**

Right knees diameter was evaluated with caliper positioned medio-laterally at the joint interline region. This evaluation was performed in moments similar to clinching threshold moments.

**RESULTS**

Results have shown that there has been hypernociception in GC without returning to baseline values, differently from what was observed for treated groups. GUP has returned to baseline levels as from EV3 and GUC as from EV4 (Table 1). There have been no significant differences when comparing among groups.

| Table 1. Values in grams, obtained with digital Von Frey filament, for the three groups, in different evaluation moments |
|---------------------|---------------------|---------------------|---------------------|
| EV1            | EV2            | EV3            | EV4            |
| CG            | 344.6±53.0a     | 246.0±38.6b     | 205.0±47.2bc    | 155.1±53.5c     |
| GUP           | 304.9±87.1a     | 157.8±66.7b     | 182.2±38.8ab    | 257.9±64.1ab   |
| GUC           | 354.6±108.3a    | 234.0±100.5b    | 202.3±55.9b     | 248.6±128.0ab  |

CG: control group; GUP: pulsed therapeutic ultrasound group; GUC: continuous therapeutic ultrasound group.

Different small letters indicate significant differences within the same group.

For edema, the three groups had similar behavior, with edema formation in EV2 without posterior reduction (Table 2).

| Table 2. Values in millimeters, obtained with the caliper for the three groups in different evaluation moments |
|---------------------|---------------------|---------------------|---------------------|
| EV1            | EV2            | EV3            | EV4            |
| CG            | 12.60±0.14a     | 14.00±0.49b     | 14.11±0.52b     | 13.60±0.42b    |
| GUP           | 12.37±0.38a     | 13.88±0.16b     | 13.68±0.21b     | 13.56±0.28b   |
| GUC           | 12.15±0.53a     | 13.93±0.69b     | 14.04±0.21b     | 13.76±0.25b   |

CG: control group; GUP: pulsed therapeutic ultrasound group; GUC: continuous therapeutic ultrasound group.

Different small letters indicate significant differences within the same group.
DISCUSSION

Ultrasound is a very popular tool to treat musculoskeletal problems, however there is the need for further studies about its beneficial effects. A survey with 207 North-American physiotherapists specialized in orthopedic physiotherapy, has shown that it is primarily used for inflammatory processes, also aiming at decreasing pain. It was observed that 75% of answers have pointed to continuous ultrasound for pain relief, with just 17.1% using 50% pulse cycles, being that most doses were between 1 and 2W/cm², in spite of hints indicating the usefulness of lower intensities for the treatment, that is, there is the need for studies evaluating lower intensities and different ultrasound release ways.

In our study, the irritation model has as major feature two nociceptive behavior stages separated by a quiescence stage around the fifth to the tenth minute after formalin injection. So, we decided to carry out the first evaluation 15 minutes after injury, avoiding evaluating in the quiescent period, with new evaluation 30 and 60 minutes after, aiming at observing nociceptive and edema behavior along 1h after injury. Evaluation with Von Frey filament provides sensitive, objective and quantifiable nociceptive measures, and edema formation measurement using the caliper is also presented by the literature. Considering that evaluators were experienced and animals were trained before evaluations, presented data are reliable, showing that there have been no changes in edema formation with the use of ultrasound, producing neither increase nor decrease, since it has been used during acute irritation stage. This is different from what has been observed by a study with tendon trauma in rats, where there has been increased edema evaluated 2 and 8h after trauma, with significant decreases 24 hours later. They also mention that nociception evaluated by clinching time, has shown hypernociception decrease 8 hours after for GUP and just 24 hours after for GUC. Although being different times, pattern was similar to our study where the two therapies used have produced nociceptive threshold decrease 20 minutes after for pulsed and 60 minutes after for continuous ultrasound.

In a different study using types of injury and evaluation similar to our study, it was observed that for continuous ultrasound animals had increased nociceptive threshold 2 hours after chemical irritation, which was changed in the group receiving naloxone before the injury, showing that a possible route for ultrasound-mediated analgesic effect could be the release of endogenous opioids after chemical irritation. It is also stressed that with parameters used, similar to the continuous group in our study, thermal ultrasound effects may be discarded, that is, the so called non-thermal effects were responsible for changes in pain threshold in both studies.

A different study, with evaluation, injury and ultrasound treatment similar to our study, aiming at evaluating cumulative effects of low-level laser, has observed that isolated application of this resource was better than laser or the association of techniques. In a previous study comparing pulsed and continuous ultrasound, in animals submitted to experimental sciatica model, ultrasound was effective to decrease pain evaluated by clinching time and, similarly to our study, pulsed ultrasound had faster analgesic results. That is, aiming at analgesic effects, not only final dose seems to be important but, since pulsed ultrasound had earlier positive results, temporal peak (SATP) may influence the action of this resource and should be the focus of future investigations. Limitation of this study was the lack of inflammatory process molecular evaluation, which also suggests further studies.

CONCLUSION

Therapeutic ultrasound was effective to decrease nociception and pulsed ultrasound had earlier results as compared to continuous ultrasound; however, both types of applications had no effect on acute edema formation and maintenance.

REFERENCES