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

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

Gladson Ricardo Flor Bertolini[1], Josinéia Gresele Coradini[2], Regina Inês Kunz[2], Bruno Pogorzelski Rocha[2], Lígia Inez da Silva[3]

*Received from State University of Western Paraná, Cascavel, PR, Brazil.

DOI 10.5935/1806-0013.20140062

ABSTRACT

BACKGROUND AND OBJECTIVES: Among electro agents ultrasound is one of the most common, however, there is insufficient evidence of the beneficial effects with the parameters currently used. The aim of this study was to compare the effect of continuous and pulsed ultrasound therapy on experimental hyperalgesia and edema in knees of Wistar rats.

METHODS: 18 rats were divided into three groups: CG- control group; GUP - treated with pulsed ultrasound 50%; and GUC - continuous ultrasound. To accomplish the lesion, animals were manually restrained and 100µL of 5% formalin solution were injected into the right tibiofemoral space. For assessment of nociception digital Von Frey filament was used on the medial side of the joint, until clinching. Edema was evaluated with mid-lateral knee caliper. Assessments occurred in the pre-injury (EV1), after 15 (EV2), 30 (EV3) and 60 (EV4) minutes of the injury. After EV2, treatment was initiated with ultrasound with 0.4W/cm^2 (SATA), pulsed or continuous.

RESULTS: The CG had hypernociception, with no return to baseline. GUP has returned to baseline as from EV3 and for continuous ultrasound in EV4. All three groups showed similar behavior for edema, with onset in EV2, without reduction.

CONCLUSION: Therapeutic ultrasound was effective to decrease nociception, and the pulsed form showed early results, however, both forms of application had no effect on the formation and maintenance of acute edema.

Keywords: Edema, Pain measurement, Ultrasound therapy.

RESUMO

JUSTIFICATIVA E OBJETIVOS: Dentre os agentes eletrotérmicos o ultrassom é um dos mais comuns, contudo, há insuficiente evidência dos efeitos benéficos com os parâmetros correntemente utilizados. Assim, o objetivo deste estudo foi comparar o efeito do ultrassom terapêutico contínuo e pulsado sobre hiperalgesia e edema experimentais em joelhos de ratos Wistar.

MÉTODOS: Foram utilizados 18 ratos, divididos em três grupos: GC - grupo controle; GUP - tratado com ultrassom pulsado 50%; e, GUC - ultrassom contínuo. Para realizar a lesão, os animais foram contidos manualmente e 100µL de solução de formalina a 5% foram injetados no espaço tíbio-femoral direito. Para avaliação da nocicepção foi utilizado o filamento de Von Frey digital, na face medial da articulação, até a retirada do membro. A avaliação do edema foi realizada com paquimetria médio-lateral ao joelho. As avaliações ocorreram no momento pré-lesão (AV1), após 15 (AV2), 30 (AV3) e 60 (AV4) minutos da lesão. Após AV2, foi iniciado o tratamento com ultrassom com 0,4W/cm² (SATA), de forma pulsada ou contínua.

RESULTADOS: Para GC, houve a presença de hipernocicepção, sem retorno aos valores basais. Para GUP houve retorno aos valores basais a partir de AV3 e para o ultrassom contínuo em AV4. Para o edema, os três grupos apresentaram comportamento semelhante, com formação em AV2, sem redução posterior.

CONCLUSÃO: O ultrassom terapêutico mostrou-se eficaz para redução do quadro nociceptivo, sendo que a forma pulsada mostrou resultados precocemente ao contínuo, contudo, ambas as formas de aplicação não tiveram efeito sobre a formação e manutenção do edema agudo.

Descritores:Edema, Mensuração da dor, Terapia por ultrassom.

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[1,2].

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

1. University of São Paulo, School of Medicine of Ribeirão Preto, Ribeirão Preto, SP, Brazil.
2. State University of Western Paraná, Department of Physiotherapy, Cascavel, PR, Brazil.
3. Federal University of Paraná, Department of Physiotherapy, Curitiba, PR, Brazil.

Submitted in September 16, 2014.
Accepted for publication in November 06, 2014.
Conflict of interests: none – Sponsoring sources: none.

Correspondence to:
Gladson Ricardo Flor Bertolini
Rua Universitária, 2069 – Jardim Universitário
85819-110 Cascavel, PR, Brasil.
E-mail: Gladson_ricardo@yahoo.com.br

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[3].

Studies have contradictory results of therapeutic ultrasound in different types of injuries, having among studied variables pain, such as impingement syndromes[4,5], ankle torsions[6], low back pain[7,8] and knee osteoarthritis[9]. However, the diversity of both clinical and research parameters is extreme[2], 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[10].

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.

Treatment protocols

Treatment was started after the second evaluation, that is, 15 minutes after hyperalgesia induction. CG has not suffered any therapeutic intervention, just simulation. Treatment consisted in transcutaneous use of ultrasound (Ibramed®) with frequency of 1MHz, 0.4V/cm² (SATA), on the knee joint interline, with slow and circular movements. GUP has received pulsed ultrasound (0.8W/cm² – SATP), with modulation of 5ms on 5ms off, that is, 50% of active cycle; and GUC has received continuous ultrasound. After last evaluation, animals were euthanized.

Statistical analysis

Data normality was checked with Kolmogorov-Smirnov test. Then, data were analyzed by unidirectional ANOVA (for comparison among groups) and ANOVA with repeated measures (comparison within groups). In all cases, significance level was 5%.

This study was carried out according to international animal experiment ethics rules, after approval by the Ethics Committee for Animal Use, UNIOESTE, under opinion 4412/2012.

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/cm^2 [2], in spite of hints indicating the usefulness of lower intensities for the treatment[1], 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[11]. 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[12], and edema formation measurement using the caliper is also presented by the literature[13]. 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[14]. 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[3]. 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[15]. 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[10]. 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

1. Warden SJ. A new direction for ultrasound therapy in sports medicine. Sports Med. 2003;33(2):95-107.
2. Wong RA, Schumann B, Townsend R, Phelps CA. A survey of therapeutic ultrasound use by physical therapists who are orthopaedic certified specialists. Phys Ther. 2007;87(8):986-94.
3. Martignano CC, Silva LI, Meireles A, Rocha BP, Rosa CT, Bertolini GR. Avaliação do ultrassom sobre a hiperalgesia e o edema em joelhos de rato Wistar e interferências de um inibidor de opioides endógenos. Fisioter Bras. 2013;14(4):289-93.
4. Yildirim MA, Ones K, Celik EC. Comparison of ultrasound therapy of various durations in the treatment of subacromial impingement syndrome. J Phys Ther Sci. 2013;25(9):1151-4.
5. Alexander LD, Gilman DR, Brown DR, Brown JL, Houghton PE. Exposure to low amounts of ultrasound energy does not improve soft tissue shoulder pathology: a systematic review. Phys Ther. 2010;90(1):14-25.
6. van den Bekerom MP, van der Windt DA, Ter Riet G, van der Heijden GJ, Bouter LM. Therapeutic ultrasound for acute ankle sprains. Eur J Phys Rehabil Med. 2012;48(2):325-34.
7. Fiore P, Panza F, Cassatella G, Russo A, Frisardi V, Solfrizzi V, et al. Short-term effects of high-intensity laser therapy versus ultrasound therapy in the treatment of low back pain: a randomized controlled trial. Eur J Phys Rehabil Med. 2011;47(3):367-73.
8. Licciardone JC, Minotti DE, Gatchel RJ, Kearns CM, Singh KP. Osteopathic manual treatment and ultrasound therapy for chronic low back pain: a randomized controlled trial. Ann Fam Med. 2013;11(2):122-9.
9. Luksurapan W, Boonhong J. Effects of phonophoresis of piroxicam and ultrasound on symptomatic knee osteoarthritis. Arch Phys Med Rehabil. 2013;94(2):250-5.
10. Ciena AP, Cunha NB, Moesch J, Mallmann JS, Carvalho AR, Moura PJ de, et al. Efeitos do ultrassom terapêutico em modelo experimental de ciatalgia. Rev Bras Med Esporte. 2009;15(6):424-7.
11. Martins MA, de Castro Bastos L, Tonussi CR. Formalin injection into knee joints of rats: pharmacologic characterization of a deep somatic nociceptive model. J Pain. 2006;7(2):100-7.
12. Vivancos GG, Verri WA Jr, Cunha TM, Schivo IR, Parada CA, Cunha FQ, et al. An electronic pressure-meter nociception paw test for rats. Braz J Med Biol Res. 2004;37(3):391-9.
13. Gould D, Yousaf N, Fatah R, Subang MC, Chernajovsky Y. Gene therapy with an improved doxycycline-regulated plasmid encoding a tumour necrosis factor-alpha inhibitor in experimental arthritis. Arthritis Res Ther. 2007;9(1):R7.
14. Bertolini GR, Silva TS, Ciena AP, Artifon EL. Comparação do ultrassom pulsado e contínuo no reparo tendíneo de ratos. Fisioter Pesq. 2012;19(3):242-7.
15. Coradini JG, Mattjie TF, Bernardino GR, Peretti AL, Kakihata CM, Errero TK, et al. [Comparison of low level laser, ultrasonic therapy and association in joint pain in Wistar rats]. Rev Bras Reumatol. 2013;54(1):7-12. English, Portuguese.