Effect of continuous therapeutic ultrasound in rabbit growth plates

Andersom Ricardo Fréz[1], Déborah Ariza[1], José Roberto Leonel Ferreira[2], Éder Paulo Belato Alves[3], Gláucia Ritter Breda[4], Lígia Aline Centenaro[4], Tiago Kijoshi Ueda[4] and Gladson Ricardo Flor Bertolini[5]

ABSTRACT

The therapeutic efficiency of ultrasound has become an indispensable tool of physical therapy treatment in cases of alteration by lesions and in many kinds of sickness. However, in pediatric cases the use of ultrasound is controversial due to possible disturbance and damage to the growth plate. The aim of this study is to find out if the continuous ultrasound presents alteration effects on the growth plate of female rabbits. Eight New Zealand female rabbits with two months of age were tested. They were treated by continuous therapeutic ultrasound with doses of 1 W/cm^2 in the lateral region of the right knee joint for five minutes, during 10 days, with an interval of two days after five applications. The left knee joint was used as a control. The histological analysis showed an alteration in the thickness of the growth plate on the treated side 24.40% bigger than in the left knee joint of the control ($p < 0.0001$). On other hand, the radiological analysis did not show any difference between the limbs. The conclusion was that the therapeutic ultrasound produced significant histological alterations in the cartilage thickness on the treated side according to the manner it was used in the experiment. Such fact suggests an acceleration in the growth plate metabolism.

Keywords: Ultrasound therapy. Growth plate.

INTRODUCTION

The ultrasound is known as a therapeutic treatment which presents clinical evidence for the use in lesions of the soft tissues with the aim to speed healing[1-3], decrease articulatory stiffness, reduce pain and muscular spasm[4], increase the colagen synthesis[5], and regeneration of the peripherical innervation[6].

However, many reports on its application in the muscular-skeletical system have been focusing in the fractures consolidation[4], presenting good results in the formation[7-8] and consolidation of the bone corn, in animal models and humans as well[7,9-10]. Moreover, the ultrasound can increase the osteoblasts density[11], stimulate the cellular matrix secretion[12-13] and of growth factors envolved in fractures consolidation[14].

All these effects make therapeutic ultrasound to be chosen for the treatment of alterations caused by lesions and many illnesses. However, there is some controversy about its use in pediatric patients, since some studies show possible disturbs and damage to the growth plate[4,15], which may lead them to a premature fusion, with consequent discrepancy in the limbs length or an uneven growth in one of the sides of the bone[16].

Being an efficient resource in many cases in the clinical practice, it is believed that further studies are need to evaluate whether its use in regions of endochondral ossification should be applied, once the literature is not conclusive in relation to the possible deleterial effects[15,17-18].

The aim of this study is to evaluate the effects of the treatment with continuous therapeutic ultrasound on the growth plate of New Zealand female rabbits' tibia during the development phase through histological analysis. Moreover, after the initial growth phase, we verify whether there are alterations in the tibia through radiological exam.

METHODS

The present work is characterized as experimental with qualitative and quantitative analysis 08 New Zealand female rabbits with 2 months of age were used. The animals were kept in cages and standardized food and drinkable water were given *ad libitum*. All of them received the therapeutic ultrasound application in the lateral region of growth plate of the right tibia and the left back paw was used as control.

The ultrasound equipment used was the Avatar II model by KLD, with 1 MHz head and Effective Radiation Area (ERA) of 4 cm^2. It was previously calibrated. The used parameters were: dose of 1,0 W/cm^2 (Spacial and Temporal Averages – SATA), during 5 min, in a subaquatic manner (immersion in water), in continuous emission, using a plastic dish without filling as a container for application to absorb the ultrasound, and treated water as conduction means. The animals had their right back limbs tricotomized in the first and sixth days of the therapy.

The procedures occurred in 10 sessions, daily conducted, with an interval of 2 days after the 5th session, simulating the procedure in the physical therapy in humans. The animals were manually held during the session.

10 days after the last session, 4 animals were sacrificed. The back limbs were desarticulated in the hips and ankles, and the femur and tibia cleaned from the soft parts, only the junction by the ligaments and the knee articulatory capsule being kept. They were all kept in 10% formol.

The tibias were separated from the femurs and transversally sawn in the diaphysary region in order to make the slides. Afterwards, the proximal segment was submitted to the histological processing. Firstly, the tibia was attached to an acid solution (nitric acid) for decalcification. Later, the tissue was soaked in paraphine, lengthwide cut and stained by the eosin hematoxylin. The mounting of three histological slides occurred through growth plate, after digital capture of the images. The height of the plates was

1. Fisioterapeuta Graduado pela Universidade Estadual do Oeste do Paraná (UNIOESTE) – Campus de Cascavel.
2. Médico Radiologista, Docente da Disciplina de Radiologia e Diagnóstico por Imagem da UNIOESTE.
3. Docente da Disciplina de Histologia da UNIOESTE.
4. Acadêmico de Fisioterapia da UNIOESTE.
5. Docente da Disciplina de Eletrotermofototerapia e Supervisor de Estágio em Fisioterapia Ortopédica e Traumatológica da UNIOESTE.

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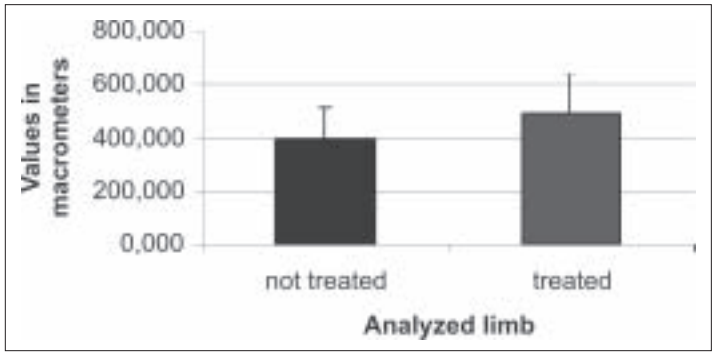
Correspondence to: Mono.
Endereço para correspondência: Gladson Ricardo Flor Bertolini, Rua Luiz Vilwock, 125, B, Nova Cidade – 85803-177 – Cascavel, PR. E-mail: gladson_ricardo@uol.com.br; gladson_ricardo@ibest.com.br

checked, that is, their thickness, and 9 measurement in the lateral, intermedium and medium regions were done. The program used for the measurements was the Image-Pro-Plus 3.0.1. The paired *t* test of the Prism 2 01 program was used for the statistical analysis.

The 4 remaining animals were evaluated through radiological exams at 4 months old, trying to observe any epiphysary alteration and/or significative discrepancy between the length of the treated limb and the control. The equipment used for the radiological exam was the Tridoros 812E model by Siemens The used parameters were: 150 mA, t = 0,02 s and kV 49, Emic Limex, and anterior-posterior incidence. The analysis was conducted by two radiology physicians, members of the Brazilian College of Radiology, both with 20 years of experience in the field. Initially, a comparative analysis of the right and left lower limbs was done. The radiologists did not have previous knowledge of which limb was the object of the study. The physicians were then notified about the methodology and the aim of the experiment and a new analysis was conducted.

RESULTS

The obtained values in the histological analysis showed that in the control side, the size of the growth plates was around 393,359 ± 117,934 μm, and, the treated side presented thickness of 489,354 ± 147,386 μm, having a significant variation of 24,40% more on the treated side ($p < 0,0001$) (graphic 1).



Graphic 1 *– Comparison between the obtained values in the histological analysis of the thickness of the plates on the not-treated side and treated side*

In the radiological analysis the medical report discriminated: soft tissues with usual attenuation of the X-rays; bone trabeculae with homogeneous distribution and density, without areas of osteopenia visible through the method; straight and regular surfaces with preserved spaces; symmetrical growth plates with usual shape and density. No radiological evidence of bone demineralization areas was found. After the explanation on the work methods, there was a new evaluation from the radiologists, who were in doubt about the plates spaces, however, they attributed such doubts to a slight positioning asymmetry.

DISCUSSION

The objective of this study was to determine whether the therapeutic ultrasound may cause alterations in the growth plate and clinically detectable alterations to the radiological exam. According to Yang *et al.*[19] and Gebauer *et al.*[20] the ultrasound stimulus to the formation of the bone corn is due to the synthesis increase of the proteins of the extracellular matrix in the cartilage, possibly altering the chondrocytes maturation and the formation of the endochondral bone. Parvizi *et al.*[12] credit the acceleration in the consolidation of fractures to the stimulation in the synthesis of proteoglycan cellular matrix. Reher *et al.*[5] credit the stimulus of bone formation to the colagen synthesis, Li *et al.*[11] credit the acceleration to the. E_2 prostaglandin synthesis, though.

Zhang *et al.*[13] applied pulsed ultrasound of low intensity for 20 min, during 7 days, in sternum bone culture of chicken embrios and concluded that the acceleration in the bone formation occurs by the increase in the condrocytes hypertophy directly in the terminal differentiation, without causing hypertrophy in the hyaline cartilage. However, Nolte *et al.*[17] using the same parameters, but in rat fetuses cells culture, observed increase in the endochondral calcification after 7th day of application, and the histological analysis presented healthy conditions, similarly to the control. They also credited the ossification to the direct effect in the osteoblasts and cartilage calcification.

Spadaro and Albanese[21] studied the effect of the low intensity ultrasound in growing rats' long bones during four weeks, with daily 20 min applications. The results showed that the ultrasound did not interfere in the femur and tibia's growth nor allowed increase of the bone mineral density.

Wiltink *et al.*[15] using metatarsus fragments of mice fetuses, treated with 1 MHz ultrasound, pulsed 100 Hz (2 ms: 8 ms), with 0,77 W/cm² (Spacial Average and Temporal Peak – SATP) observed increase in the length of the proliferative zone of the growth cartilage and lack of alterations in the hypertrofic cartilage region, concluding that there was proliferation without stimulation of the cellular differentiation.

Rabbits were chosen for this work due to their suitable size and easy positioning of the ultrasound head. The tibia proximal region was selected due to its skin localization, making the positioning of the transductor easy, and, the ultrasound application way was subaquatic due to greater easiness for handling of the head in the animal's back limb region. Moreover, there are few acoustic problems between water and the soft tissue, leading to little reflexion[22].

Deleterious action of the ultrasound may be observed in this present study, once its action over the growth plate was seen, increasing its thickness and leading to evidence of increase in the metabolism in the plate with probable cellular proliferation.

These data agree with the histological findings by Lyon *et al.*[4] who using continuous ultrasound, 1 MHz, 2,2 W/cm², for 20 min, in New Zealand female rabbits, obtained a significant increase in the cartilage thickness. Nevertheless, alterations in the metaphysis contour, undistinctive epiphysis line and tibia plateau in curved shape causing varo angulation, occurred in these authors' radiological analysis, being the last one not pointed in the actual study.

The radiologists did not find suggestive or characteristic of possible visible bone alterations radiological signs through the method. During the reevaluation the physicians were uncertain about the epiphyses spaces, which was credited to the slight asymmetry of positioning.

Correlating to the clinical practice, the results point that the therapeutic ultrasound applied with the present parameters, should not be used for treatment in sites with open growth plate, since the histological exam showed significant alterations, presenting epiphyses stimilation. Although the reports presented in the radiological evaluation did not point any visible alterations, the continuation of these exams with the adult animal could present deformities, a fact neglected in the present study.

In the present study, the small number of animals used for the histological and radiological analysis was seen as a restriction. Besides that, other ways of the ultrasound presentation were not evaluated, namely variations in the density of exit power, in the base frequency (1 or 3 Mhz) and in the way of giving (continuous or pulsed).

CONCLUSIONS

The therapeutic ultrasound of 1 MHz, with dosage in 1 W/cm^2 SATA, whenever applied in the continuous way does not produce alterations tangible through the radiological exam in female rabbits at 4 months old. On the other hand, when a histological analysis is done, significant alterations in the cartilage thickness on the treated side are observed, suggesting an acceleration in the plate metabolism. New studies are necessary for further knowledge on the morphological and histological responses on the growth plate. Furthermore, considering correlation between studies in animal models and human models, a safe dosage for pediatric patients, guaranting the lack of possible deleterial effects, should be tried.

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