EFFECTS OF THERAPEUTIC ULTRASOUND ON LONGITUDINAL GROWTH OF THE FEMUR AND TIBIA IN RATS

ALFREDO ALCANTARA BARRETO[1], ROMI KAWASAKI ALCANTARA BARRETO[2], HAMILTON DA ROSA PEREIRA[1], WILLIAM SAAD HOSNNE[1]

ABSTRACT

Objectives: To determine the effects of ultrasound therapy on the femur and tibia growth in young rats. Method: Four-week-old male Ratus Norvegicus totaling 115 animals, divided into four groups, were submitted to ultrasound therapy (0.8 MHz, fixed tube head, continuous pulse, for 10 minutes, once a day, ten times) on the medial face of the right knee, with powers of 0.0 W/cm2 (group G1), 0.5 W/cm2 (group G2), 1.0 W/cm2 (group G3), and 1.5 W/cm2 (group G4). Histological slides of the epiphysis, growth plate and metaphysis and the femoral and tibial length measurements were studied in the sixth, thirteenth and twenty-sixth weeks of life. The data were submitted to factorial analysis of variance according to a one-way layout. Results: No statistically significant bone growth alteration was established between any of the three treated groups and the control group. However, alterations in femoral and tibial growth suggesting a decrease in G4 in relation to G2 and G3 were noted. In G4, histopathological alterations, such as cellular necrosis and post-necrosis bone neoformation were found. Conclusion: According to this study, no statistical evidence of bone growth stimulus or inhibition resulting from the application of ultrasound therapy was found when comparing the treated groups with the control group. Histological alterations regarded as pathological were only observed in G4. Also, smaller significant bone growth was found in G4 compared to G2 and G3. Level of Evidence: Level II, cross-sectional study.

Keywords: Ultrasound therapy. Bone development. Epiphyses. Rats.

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INTRODUCTION

Alongside beneficial effects such as selective heating of the tissues,[1,2] undesirable effects can occur with the use of ultrasound, such as cavitation effect;[3-6] heatstroke in poorly vascularized tissues, hyperemia, inflammatory responses characterized by increase in vascularity, edema and tissue necrosis,[7] rendering therapeutic use uncertain in some situations, such as vascular deficiency, infections, on the nerve tissue and epiphyseal plate.[8-10]

The biological studies performed used a broad range of frequency and type of pulse, power, number and time of each ultrasound application, with animals of different ages and species (rats and rabbits mostly, but also dogs and sheep) and human beings. The results of the studies, although with some differences,[11] show vascular alterations,[12] stimulus to the healing of skin ulcers,[13] of tendons,[14] in the formation of fracture callus,[15,16] but not in bone neoformation,[17] with few more recent studies on its effects on longitudinal bone growth and on the epiphyseal plate.[18-20]

This diversity makes it hard to compare the data and to have a clear understanding of the biological effects of ultrasound, so much so that Wells[3] considered the scientific knowledge about the biological bases of its use "disappointing" and Haar[4] and Leighton[6] considered the need for more investigations in this respect.

We decided to prepare this study, aiming to investigate the effects of different powers of therapeutic ultrasound on longitudinal bone growth of the femur and tibia, under conditions similar to those employed in clinical therapeutics, using common use

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1–Surgery and Orthopedic Department – Orthopedics and Traumatology Discipline - Faculdade de Medicina de Botucatu – UNESP, Brazil.
2–Rehabilitation Service of SESI – São Paulo, Brazil.

Study conducted at the Surgery and Orthopedic Department of Faculdade de Medicina de Botucatu da Universidade Estadual "Julio de Mesquita Filho" – UNESP – Botucatu (SP), Brazil. Mailing Address: Departamento de Cirurgia e Ortopedia – Disciplina de Ortopedia e Traumatologia - Faculdade de Medicina de Botucatu – UNESP, Campus de Rubião Junior – CEP: 18618-970 – Botucatu (SP), Brazil. E-mail: alalcbar@fmb.unesp.br.

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equipment, and observing bone growth until adult age.

We decided to use the rat as experimental subject of several studies on epiphyseal plate anatomy and physiology.[21,22] Rats have a high growth rate until they reach sexual maturity in the 13th week of life, and although the epiphyseal plate remains open throughout their lives, there is minimum growth after the 26th week of life.[21]

MATERIAL AND METHOD

The subjects were 115 four-week-old Norvegicus rats, divided into four groups, submitted to various ultrasound powers (Table 1), and monitored up to the 26th week of life.

Table 1. *Identification of the groups and number of animals.*

Group	G1	G2	G3	G4	n
Power	0.0	0.5	1.0	1.5	
Number of animals	30	28	27	30	115

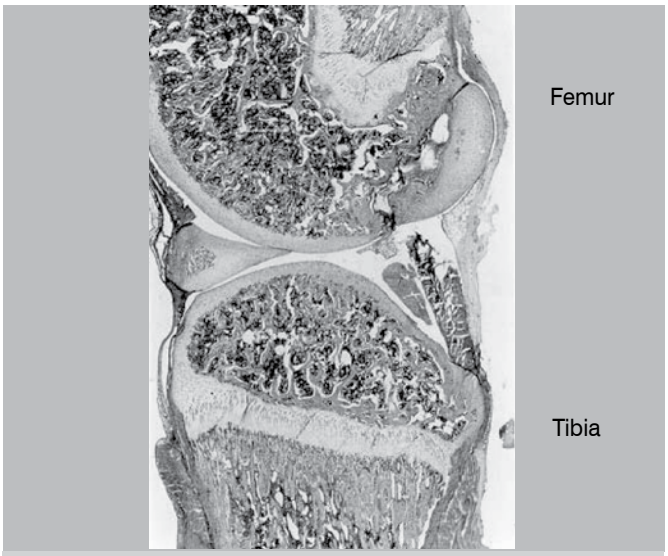

Figure 1. *Panoramic section showing the epiphyseal plates, distal femoral and proximal tibial metaphyses and epiphyses .*
(Masson – AO 5x)

The ultrasound was applied with the animals under anesthesia, using a commercial T.U.R. apparatus at 0.8 MHz of frequency, at the powers of 0.0 W/cm^2, 0.5 W/cm^2, 1.0 W/cm^2 and 1.5 W/cm^2. The applications were daily, lasting for 10 minutes each, five times a week and for two consecutive weeks. The fixed US head was placed on the medial side of the right knee, according to Buchtala's water balloon method,[7] trichotomized previously.

Control group G1 was anesthetized and the US head was applied with the apparatus turned off.

Ten animals from each group were chosen by lot to be sacrificed at the following times of the experiment: T2 - six weeks of life (day following the 10th ultrasound application), T3 - at 13 weeks of life and, T4 - at 26 weeks of life. No samples were taken on T1, day of the first ultrasound application.

At each of these times, the femur and tibia were extracted from the right and left sides of each sacrificed animal, and their respective lengths were established in hundredths of millimeters with the use of a caliper. The results of the treated groups were compared individually with the control group and to one another, and the right side was compared to the left.

After the measurements of length, the fragments were fixed in formaldehyde, decalcified (Bouin's solution) and arranged on slides, stained by Mason's method, for histopathological examination. The histological sections were prepared so as to enable the visualization of the metaphyses, epiphyseal plates and epiphyses, distal to the femur and proximal to the tibia (Figure 1), and the epiphyseal disc was investigated in search of histological lesions.

Statistical method

The study of longitudinal bone lengths of the femur and of the femur-tibia assembly on the right side was performed at each time comparing each group with all the others, with statistical verification by analysis of variance in completely randomized factorial design– (Snedecor and Cochran[23]) testing the hypotheses of group effect for the set of times and group effect at each time. The same statistical analysis was used to compare the right and left sides where differences were verified among times with the group fixed. In all the conclusions the significance level considered was 5%.

RESULT

General clinical aspects

In 14 animals from group G4 (1.5 W/cm^2) we discovered the occurrence of hyperemia and skin ulcers, and in one of these animals necrosis of the musculature, joint capsules and ligaments, with subluxation of the knee, necrosis and bone reabsorption were observed. In six animals from group G3 (1.0 W/cm^2) we found claudication, hyperemia and discreet edema in the right knee. No clinical alteration was found in the animals from group G2 (0.5 W/cm^2) and G1 (control).

HISTOLOGICAL ASPECTS

We did not find any microscopic alterations that could be considered pathological in the animals from groups G1, G2 and G3. We found areas of bone hemorrhage and cellular proliferation (Figures 2 and 3), irregularities and gaps in the epiphyseal plate proliferation zone, disorder in the palisade arrangement of the chondrocytes and bone neoformation (Figure 4), similar to those described by De Forest *et al*.[8] and Ito[9] that considered them pathological, in animals from all the groups, on the right side (treated), (but also on the left side and in group G1 not submitted to ultrasound) and at all the times.

The presence of bone necrosis, granulation tissue, neutrophils and bone neoformation by regeneration (Figure 5) were observed on the treated size in group G4 at T2, with one or more of

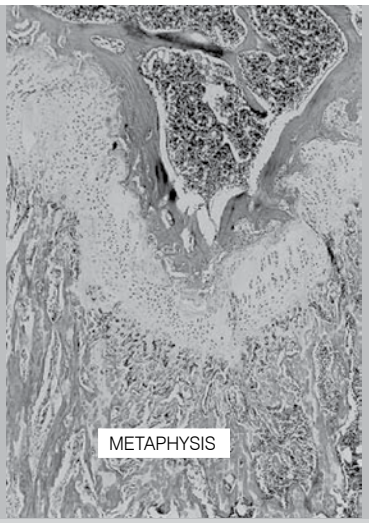

Figure 2 – *Group G1 (control) – M3 – epiphyseal plate: cellular and hypercellular areas, hemorrhage focuses and irregularities in the palisade arrangement. (Masson AO 13.2x)*

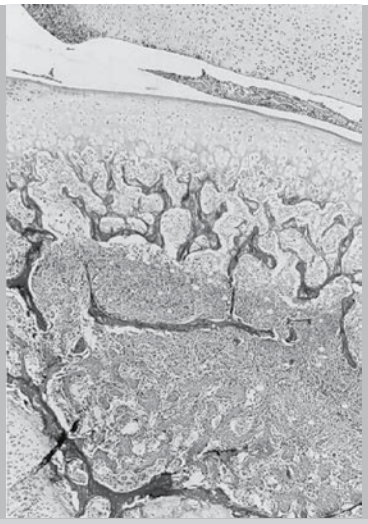

Figure 5. *Group G4 (1.5 W/cm^2) – right side – M2 – epiphysis: bone neoformation by regeneration and neutrophils. (Masson – AO 10x)*

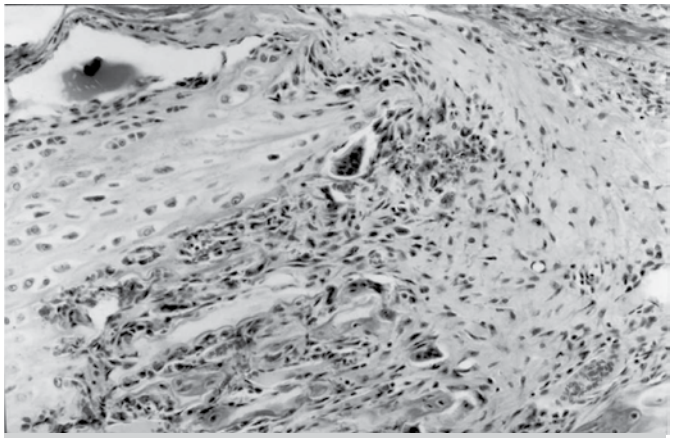

Figure 3. *Group G2 (0.5 W/cm^2) –Control side - M2 – epiphysis: area of bone resorption, osteoclasts and new bone formation. (Masson – AO 50x)*

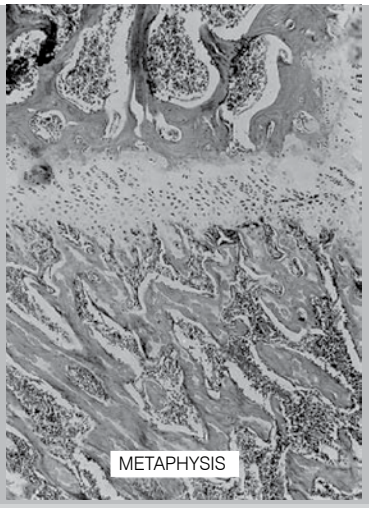

Figure 4. *Group G2 (0.5 W/cm^2) – left side – M3 – epiphyseal plate: disorder in the proliferation zone. Hemorrhage focuses in the spinal cord. (Masson - AO 20x)*

these alterations having been noticed in almost all the animals. Although bone neoformation was also found on the left side, on the right side we observed bone neoformation by regeneration after bone necrosis. In one animal we observed destruction of the epiphysis, epiphyseal plate and tibial metaphysis, granulation tissue and a large quantity of neutrophils.

In general, the metaphysis, epiphysis and epiphyseal plate both of the femur and of the tibia, appeared histologically normal, except in animals from group G4 and to a lesser degree from group G3.

Bone length

In the group of animals submitted to 1.5 W/cm^2 (G4) there was a statistical tendency toward less femoral growth than in treated groups G2 and G3, but not in comparison to control group G1. (Table 2) When the tibia was included in the measurement the statistical behavior was similar. (Table 3)

In the comparison between right and left sides of the femur

Table 2. *Length of femur–Completely randomized factorial analysis of variance.*

Hypotheses tested	Statistics calculated	Remarks
H02: Group effect for the set of times	critF = 2.71 calcF = 6.47	G2 = G3) > G4 G1 does not differ from the others
H04: Group effect at each time	msd = 312	T2: G1 = G2 = G3 = G4 T4: (G2 = G3) > G4 G1 does not differ from the others

Critical F (critF), calculated F (calcF), least significant difference (lsd).

Table 3. *Length of limb - Completely randomized factorial analysis of variance.*

Hypotheses tested	Statistics calculated	Remarks
H02: Group effect for the set of times	critF = 2.71 calcFc = 7.68	G2 = G3) > G4 G1 does not differ from the others
H04: Group effect at each time	msd = 572	T2: G1 = G2 = G3 = G4 T3: G3 > G4 only difference T4: (G2 = G3) > G4 G1 does not differ from the others

critical F (critF), calculated F (calcF), least significant difference (lsd).

(Table 4) we can observe greater growth of the right side for a given period of time in G2. In G3 there was no difference over time. In G4 there was less growth of the right side at first, partially compensated at T4. When the tibia measurement is included (Table 5), we notice the same previous behavior for the groups in G2 and G4, but in G3 we observe, at T4, that the right side is significantly larger than the left side.

Table 4. *Length of femur – comparison between right and left sides.*

Hypotheses tested	Statistics calculated	Remarks
Difference between sides G2 Right and left	T2: t = 0.52p > 0.50	right = left
	T3: t = 3.00 p < 0.01	right>left
	T4: t = 0.13p > 0.50	right = left
G3	T2: t = 1.66p > 0.10	right = left
	T3: t = 1.07p > 0.30	right = left
	T4: t = 0.43 p > 0.50	right = left
G4	T2: t = 3.09p < 0.01	right<left
	T3: t = 1.29 p > 0.20	right = left
	T4: t = 1.91 0.05 < p < 0.10	right<left

Table 5. *Length of limb– comparison between right and left sides.*

Hypotheses tested	Statistics calculated	Remarks
Difference between sides G2 right and left	T2: t = 0.59 p > 0.50	right = left
	T3: t = 2.66 p < 0.05	right>left
	T4: t = 1.15 p > 0.20	right = left
G3	T2: t = 0.99 p > 0.30	right = left
	T3: t = 1.47 p > 0.10	right = left
	T4: t = 2.42 p > 0.05	right>left
G4	T2: t = 6.45 p < 0.001	right = left
	T3: t = 1.29 p > 1.15	right<left
	T4: t = 1.96 0.005 < p< 0.10	right< = left (tendency)

DISCUSSION

The incidence of clinical complications in the knee found in G3 (1.0 W/cm^2) and G4 (1.5 W/cm^2) was similar to the findings of Buchtala[7] and Ito,[9] who applied ultrasound at powers of 2.0 W/cm^2 or above.

The occurrence of some of the alterations described in literature, such as: necrosis, reabsorption, periostitis, bone proliferation and rarefaction; closing of the epiphyseal plate and separation of the epiphysis; decrease and increase of osteoblasts and osteoclasts; as observed by Buchtala[7], De Forest *et al*.[8] and Ito[9], was only encountered in this study in the group treated with 1.5 W/cm^2 (G4) and enables the conclusion that these effects are related to power as suggested by Lehmann,[3-10] although it is not observed using powers of 0.77 W/cm^2 or lower.[18-20]

Since many of the histological descriptions considered pathological by literature were found in our study, both on the right side and on the left side (untreated) and also in the control group not submitted to ultrasound, we suggest that some histological findings of studies from literature, besides being pathological alterations, are products of the techniques used to obtain segments and to prepare slides and/or represent different aspects of normality.

Based on the statistical analysis it is concluded that ultrasound did not produce an effect of inhibition or stimulus on bone growth of the femur or tibia, in comparison with the control group. However G4 (1.5 W/cm^2), at T4, was significantly smaller than G2 (0.5 W/cm^2) and G3 (1.0 W/cm^2), suggesting a growth inhibiting effect in G4 and/or stimulating effect in G2 and G3.

When we compare the treated and untreated sides, we observe in G2, both for the femur and for the limb, a greater difference of the right side at T3, which disappears at T4, suggesting temporary stimulus of bone growth (Tables 4 and 5). In G4 the right side was smaller than the left at T2, partially compensated at T4. In G3, at the end of the experiment at T4, the right limb is statistically larger than the left (Table 5), suggesting growth stimulus.

CONCLUSION

No significant statistical alteration was observed, at the end of the experiment, which could determine an effect of inhibition or stimulus on bone growth of the femur or of the limb between the treated groups and the control group.

It could be considered that, in comparing the groups of 0.5 and 1.0 W/cm^2 with the group of 1.5 W/cm^2, there is a tendency toward femoral growth stimulus in the first two groups and/or inhibition in the last group, but this effect was not significant in relation to the control group. Further studies are necessary for better conclusions.

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