Atrophic pseudoarthrosis. Effect of extracorporeal shock waves application in an experimental model in tibia of rabbits

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Abstract

Introduction: The effects of extracorporeal shock waves have been investigated in human osteoblasts, fracture lines, non-unions (pseudoarthroses) and periosteal cells. The best results of the treatment of non-union with extracorporeal shock waves haven been documented in hypertrophic non-union. The aim of this study was to investigate the effect of the therapy with extracorporeal show waves on "atrophic" non-union lines generated in rabbits' tibial bones.

Methods: We established three groups: A, fractured bones subject to extracorporeal shock waves; B ("control"), fractured bones not subject to shock waves and C, not-fractured bones (right legs). We treated white skeletally mature 37 rabbits (cuniculus NV) from New Zeeland. We practiced 20-mm periosteal cauterization with bipolar electrocautery in both (proximal and distal) bone stumps. Afterwards we applied extracorporeal shock waves in just one session. We carried out H-E stain. We made biomechanical analyses with a "3 point" loading method. We studied the maximal load applied and the elasticity module for each group.

Results: Histological analyses allowed us to register bone healing signs —periosteal and endosteal bone calluses— considerably bigger in group A-rabbits' tibial bones **(treated with extracorporeal shock waves) than those in the "control" group B.**

Conclusion: In an original experimental model of atrophic non-union generated in rabbits' tibial bones by electric cauterization, we registered significant radiographic and histological changes after making an intervention of the non-union line with ESWT.

Key words: Non-union (pseudoarthrosis); extracorporeal shock waves; tibial bone; rabbits.
Level of evidence: II

Seudoartrosis atrófica. Efecto de la aplicación de ondas de choque extracorpóreas en un modelo experimental en tibia de conejos

Conflict of interests: The authors have reported none.

ISSN 1852-7434 (online) • ISSN 1515-1786 (printed) • http://dx.doi.org/10.15417/706

Resumen

Introducción: Los efectos de las ondas de choque extracorpóreas se han investigado en osteoblastos humanos, focos fracturarios, seudoartrosis y células periósticas. Los mejores resultados del tratamiento de la seudoartrosis con ondas de choque extracorpóreas se han documentado para seudoartrosis hipertróficas. El objetivo de este estudio fue investigar el efecto de la terapia con ondas de choque extracorpóreas sobre un foco de seudoartrosis "atrófica" generado en tibia de conejo.
Métodos: Se establecieron tres grupos: A, fracturados sometidos a ondas de choque extracorpóreas; B ("control"), fracturados no sometidos a ondas de choque y C, no fracturados (pierna derecha). Se trataron 37 conejos (cuniculus NV) blancos y esqueléticamente maduros de Nueva Zelanda. Se practicó la cauterización del periostio con electrobisturí bipolar en una extensión de 20 mm, en ambos muñones óseos (proximal y distal). Luego se aplicaron ondas de choque extracorpóreas en una sola sesión. Se realizaron tinciones con hematoxilina-eosina. Se efectuó el análisis biomecánico con un método de carga a "3 puntos". Se estudiaron la carga máxima aplicada y el módulo de elasticidad para cada grupo.
Resultados: El estudio histológico permitió registrar signos de consolidación –callo fracturario perióstico y endostal– considerablemente mayores en las tibias de los animales del grupo A (tratado con ondas de choque extracorpóreas) que en las del grupo B "control".
Conclusión: En un modelo experimental original de seudoartrosis atrófica generada por electrocauterización en tibia de conejos, se registraron cambios significativos radiográficos e histológicos luego de la intervención del foco mediante ondas de choque extracorpóreas.

Palabras clave: Seudoartrosis; ondas de choque extracorpóreas; tibia; conejo.
Nivel de Evidencia: II

Introduction

The experimental models usually assembled to generate non-union (pseudoarthroses) have been based on: a) focal instability due to macro-movement,[1] b) interfragmentary diastasis[2-5] and c) focal interposition.[6] The model of non-union in rabbits based on para-fracture cauterization[7] mimics the characteristics of an "atrophic" non-union.

The effects of extracorporeal shock wave therapy (ESWT) have been investigated in: human osteoblasts,[8] fracture lines,[9] non-unions[10] and periosteal cells.[11] The best results in the treatment of non-union with ESWT haven been documented for "hypertrophic" non-unions.[11-13]

The aim of this study was to investigate the effects of ESWT on "atrophic" non-union lines generated in rabbits' tibial bones.

Materials and Methods

The Research Committee at the Austral University Hospital approved the realization of this study. We established three groups which were organized the following way:

Group A: fractured rabbits subject to ESWT
Group B ("control group"): fractured rabbits not subject to ESWT
Group C: non-fractured rabbits (right leg)

Preparation of the animals

We treated 37 white and skeletally mature (cuniculus NV) rabbits from New Zeeland, all of them females, who weighted 2660 g on average and whose age was average 2-month old. All the procedures were carried out under general anesthesia using ketamine (22 mg/kg); pre-anesthesia was carried out using midazolam (2 mg/kg) and atropine (0.04 mg/kg). All drugs were administered intramuscularly.

Intervention in the fracture line

We carried out anterior longitudinal surgical bone exposure in the left rabbits' legs and practiced transversal mid-dyaphiseal osteotomy in the tibial bone using a 10-mm thick cutting blade powered by an oscillating saw (Stryker NR). Afterwards, we carried out 20-mm periosteal cauterization with bipolar electrocautery in both (proximal and distal) bone stumps.

We performed intramedullary osteosynthesis by retrograde 2.5-mm non-threaded sharp-ending wires.

The antibiotic prophylaxis protocol included ciprofloxacin (5 mg/kg) during 48 h after the surgery. We administered ketorolac (2 mg/kg) as pain killer. Specimens were kept in conditions that met the international recommendation patterns for in vivo animal experimentation (vet assistance, individual cages, constant temperature and humidity, food and water supply, and day-to-day cleaning.

Radiographic evaluation

In postoperative weeks 2, 4 and 10 we got AP and lateral X-rays (Figure 1) of the studied leg, including knee and ankle, using GE Compax equipment and a Kodak minR2000 chassis with dual-emulsion mammographic film.

ISSN 1852-7434 (online) • ISSN 1515-1786 (printed)

We documented: a) alignment, b), possible signs of mobility at the level of the fracture line, c) focal morphology (and possible hypertrophic looks), and d) the morphologic behavior of the periosteum.

Histological evaluation

Ten weeks after performing osteotomy, we sacrificed the animals by phenobarbital sodium injection and sent three samples to undergo histological analysis. The pieces were fixed in V/V 10% formaldehyde adjusted at pH=7 with phosphate buffer (Biopur Diagnostics, Argentina) and processed with H-E (hematoxylin-eosin) stain. The samples were analyzed by the same observer, who was specialized in the histopathology of the muscle-skeletal system and who ignored the origins of and the treatment that the analyzed samples had been subject to.

Application of shock wave therapy

The animals were randomly distributed between groups using envelopes previously closed and sealed. As the intervention was blind to the researcher, randomization between groups A and B was carried out by the "permuted blocks" method.

The animals in group A underwent midazolam-neuroleptoanesthesia during the ESWT.

The ESWT was applied in only one session with a shock wave generator—the Model HTL' XXI Healthec SRO with radioscopic system of impact centralization. We applied 2000 impacts of ESWT with 10-kV-intensity. The topographic distribution was applied symmetrically to both (proximal and distal) stumps in the fracture line in the intervened tibial bone, and also in such insertion limits of the periostetal stump (Figure 2).

Four specimens were excluded from the ESWT because they showed radiographic signs of bone healing before undergoing the treatment that we had set out.

Biomechanical evaluation

We carried out biomechanical analyses using a "3 points" loading method. Eighteen samples were subject to biomechanical evaluation: seven of the group A, three of the group B, and eight of the group C. Wires were re-

Figure 1. Tibial bone lateral X-ray. Experimental atrophic non-union.

Figure 2. Application of extracorporeal shock waves in one sample.

moved before the biomechanical study. The pieces were studied in a TAG: 182 model Instrom NR "universal trials machine". They were displayed between two 19-mm diameter cylindrical holders with a 75 mm-separation between them, oriented concentrically with the compression awl. We then flexed the samples with a 19-mm-diameter/ 5 mm/min-speed cylindrical awl (Figure 3). We studied the maximal load applied and the elasticity module for each group.

Statistical analysis

The statistical analysis was carried out using the version 4.0-GraphPad program. We carried out a one-way analysis of variance and a Bonferroni t test comparing the three groups to each other with p<0.05. Data are shown as average ± standard deviation.

Results

Histopathology

The histological analysis allowed us to register bone healing signs—periosteal and endosteal bone calluses—considerably bigger in the group A-rabbits' tibial bones (treated with ESWT) than those in the "control" group B (Figures 4 and 5).

Biomechanics

All the specimens showed a typical displacement-load curve characterized by three stages: 2) initial "non-linear" response, b) ascending curve and c) curve abrupt descent ("deficient" response at the skeletal solutions of continuity).

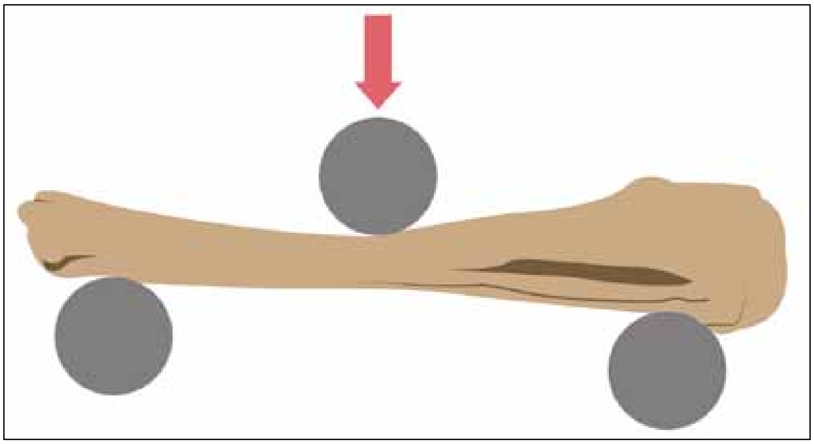
The results of the maximal load and the elasticity modules are schematized in Tables 1 and 2. The maximal load in the group of the healthy tibial bones (group C) proved to be higher than that in groups A (p<0.001; t: 7.988) and B (p<0.001; t: 6.599).

The balance between groups A and B did not evidence significant results (p>0.05; *t*: 0.4827) making allowances for the size of the samples that we used.

The elasticity module evaluated in group C showed significant changes as compared to groups A (p<0.001; *t*: 6.465) and B (p <0.001; *t*: 5.383). There were no differences in elasticity modules between groups A and B (p >0.05; *t*: 0.4326).

Figure 4. Signs of complete radiographic bone healing (periosteal and endosteal bone callus) in sample # 3 in group A.

Figure 3. Biomechanical test with a "3 points" load method.



ISSN 1852-7434 (online) • ISSN 1515-1786 (printed)

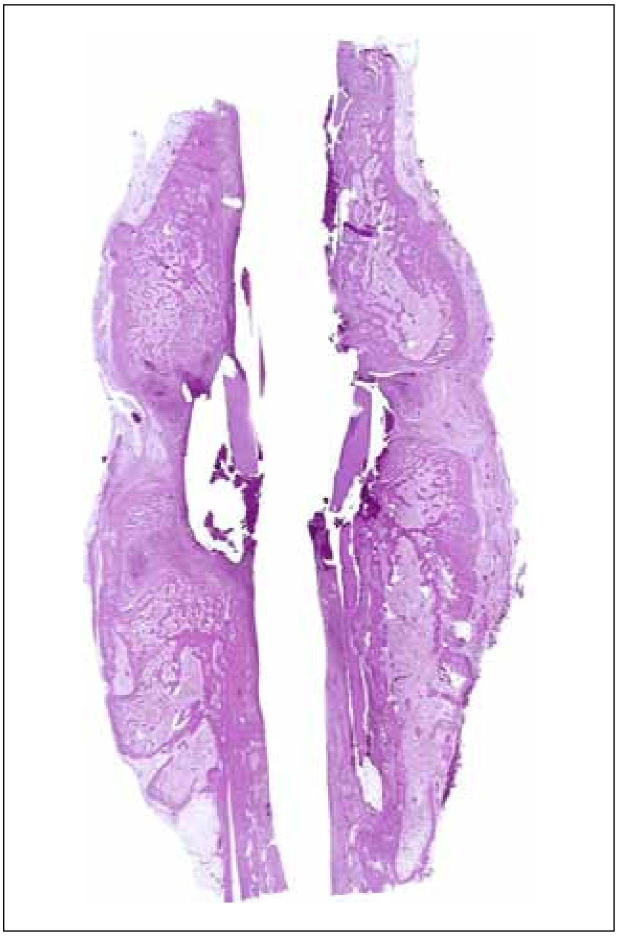


Figure 5. Signs of H-E bone healing (periosteal and endosteal bone callus) in sample # 3 in group A.

Table 1. Comparative group results of elasticity modules.

	t	p
Group C vs. Group A	6.465	<0.001
Group C vs. Group B	5.383	<0.001
Group A vs. Group B	0.4326	>0.05

Table 2. Comparative group results of the different maximal loads they were subject to

	t	p
Group C vs. Group A	7.988	<0.001
Group C vs. Group B	6.599	<0.001
Group A vs. Group B	0.4827	>0.05

Discussion

There are reports on diverse non-invasive therapeutic procedures for the treatment of non-union[14] (ultrasound, electromagnetic waves, electrical stimulation and mechanic immobilization).

ESWT is an emergent procedure worldwide; there are plenty of bibliographic sources that back its indication in diverse conditions of the muscle-skeletal system, not only at the level of bone tissues but also at that of soft tissues.[9,15]

There are reports on in vivo experimental models (in animal species) which show:

a) Favorable effects on bone healing (neovascularization, formation of new cortical bone, and promotion of BMP-like growth factors)[16] and

b) Unfavorable effects both on the mechanical stability of the affected bone and on bone marrow necrosis.[17]

Some of the most relevant experimental models of atrophic non-union are:

-Rats' tibial bones with focal diastasis kept by external fixation[2]

-Rabbits' tibial bones subject to interfragmentary diastasis[18]

-Rats' femoral bones with fracture subject to electrocautery[7]

For this study we chose a model of rabbits' tibial bones on the basis of:

-The similarities between the rabbits' sural area and the human leg (topographic distribution of soft and bony tissues)

-The size of the animal

-The dimension of the bone pieces

-The prevalence of non-union in human tibial bones

-The better dispersion of shock waves due to the lesser soft tissues coverage.

Retrospective clinical studies have shown satisfactory results with shock waves treatment in hypertrophic non-union, but worse results in the treatment of the non-union of the atrophic type.[13,14]

Histological and immunofluorescence studies on atrophic non-union show an increase in vascularization, nervous stumps at focal level and growth factors.[3,4,19] Along this lines, the hypothesis this study is based on consisted of not only such histological observations but also the proved effects of ESWT on bony tissues.

While performing this investigation we observed significant radiographic and histological changes, although they showed scarce correlation with the biomechanical study of the samples. In spite of the numerous publications that include the histological analysis of the samples, there are just few which develop an experimental model with biomechanical evaluation.

Conclusion

In an original experimental model of atrophic non-union generated in rabbits' tibial bones by electric cauterization, we registered significant radiographic and histological changes after making an intervention of the non-union line with ESWT.

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ISSN 1852-7434 (online) • ISSN 1515-1786 (printed)