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Effectiveness of Russian current in bone regeneration process in rats

Efetividade da corrente russa no processo de regeneração óssea em ratos

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Abstract

Introduction: Russian current is an electric current of average frequency that is able to restore the properties of skeletal muscle at a low treatment cost. It is essential to know the effects of Russian current in bone tissue, since electromagnetic energy could be an efficient and low cost method to treat bone disorders. **Objective**: The aim of the study was to evaluate the effectiveness of Russian current in the consolidation of tibia fracture in adult rats. **Methods**: 24 adult male Albinus Wistar rats wereused. The animals were divided randomly into two groups: control group (CG), composed of 12 animals, and Intervention Group (IG) consisting of 12 animals, both groups were submitted to osteotomy (proximal medial surface of the tibia). The IG underwent an electrical stimulation protocol with Russian current, while the CG did not undergo any kind of intervention. Euthanasia was performed in three animals of each group on the following days: 5, 10, 20, and 30 days of treatment. **Results**: The results suggested higher primary ossification, intense osteogenic activity, and increased thickness of the periosteum, characterizing more advanced ossification and a greater

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presence of trabecular bone marrow in rats in the group subjected to the treatment. In this way, we can assign one more beneficial effect to interventions with Russian current, for the treatment of postfracture rehabilitation. **Conclusion**: In both groups the bone tissue repair process occurred, but in the electrically stimulated group the osteogenesis process was more advanced.

Keywords: Fracture Healing. Rats. Physical Therapy Specialty. Electrotherapy.

Resumo

Introdução: *A corrente russa é uma corrente elétrica de média frequência que é capaz de restabelecer as propriedades do músculo esquelético a baixo custo de tratamento. É imprescindível conhecer os efeitos da corrente russa no tecido ósseo, visto que a energia eletromagnética pode ser uma forma eficiente e de menor custo para tratar alterações ósseas.* ***Objetivo****: Avaliar a eficácia da corrente russa na consolidação de fratura experimental de tíbia em ratos adultos.* ***Métodos****: Foram utilizados 24 ratos machos adultos Albinus Wistar. Os animais foram distribuídos de forma randomizada em dois grupos: Grupo Controle (GC), composto por 12 animais e Grupo Intervenção (GI) compostos por 12 animais, ambos os sofreram osteotomia (face medial proximal da tíbia). O GI foi submetido a um protocolo de eletroestimulação com corrente russa, enquanto o GC não sofreu nenhum tipo de intervenção. Foi realizada a eutanásia de três animais de cada grupo nos seguintes períodos: 5°, 10°, 20° e 30° dia de tratamento.* ***Resultados****: Os resultados sugeriram maior ossificação primária, intensa atividade osteogênica e aumento da espessura de periósteo, caracterizando assim uma ossificação mais avançada com maior presença de trabéculas na medula óssea no grupo de ratos submetidos ao tratamento. Desta forma, pode-se atribuir mais um efeito benéfico nas intervenções com corrente russa, para o tratamento de reabilitação pós-fratura.* ***Conclusão****: Em ambos os grupos analisados ocorreu processo de reparação tecidual ósseo, porém no grupo eletroestimulado o processo de osteogênese foi mais avançado.*

Palavras-chave: *Consolidação da Fratura. Ratos. Fisioterapia. Eletroterapia.*

Introduction

It is known that bone is an adaptive tissue, capable of being modified according to its structure and function, responding to mechanical forces and metabolic demands. The bone deposition process can be regulated in proportion to the strain imposed on it. Thus, bone deformation is able to produce direct electrical current, which can stimulate bone formation (1, 2).

Treatments for impairments in bone structural integrity are widely discussed in the literature, as well as the use of electrical stimulation as a therapeutic resource (2 - 6). However, the biomechanical responses of bone tissue to electro-stimulation are still controversial, since the bone response is different depending on the method and parameters applied, factors that lead to extensive debate on this issue (4, 6, 7, 8).

It is known that electrical stimulation, in different modalities, is capable of promoting accelerated bone mineral production through increased cytoplasmic calcium concentration, and promoting increased proliferation and osteoblast differentiation mediated by increased synthesis of nitric oxide (9, 10). Thus, the use of electric currents in bone tissue interventions has the potential effect of accelerating the osteogenesis process (7, 8, 11).

The application of low-frequency electrotherapy can provide biological effects in different tissues, and provide osteoblast proliferation, and an increase in extracellular matrix production, and therefore increase osteogenesis (12). It is believed that average frequency currents have similar effects (13). Russian current (RC) is an average frequency sinusoidal alternating current (biphasic) of 2,500 to 5,000 Hz, which is capable of restoring the properties of skeletal muscle (13, 14). The RC is able to reach the deep muscular structures due to its average frequency. Muscle responses have a central and peripheral origin and muscle strengthening results are retained even after the suspension of applications (11, 12). Thus, this type of current is widely applied in cases of compromised tropism and muscular strength, and its effectiveness in these cases is well established (15 - 18). However its effects on bone tissue with loss of integrity are still unknown.

Studies on body mechanics, including bone tissue, are essential for the improvement of therapeutic interventions. The combination of biomechanical and histomorphological knowledge of biological tissues is extremely important for the elucidation of injury processes and alterations in adaptive capacity.

Thus, it is essential to know the effects of RC on bone tissue, since electrical energy could be an efficient and low cost treatment for bone disorders, as well as to deepen and disseminate knowledge on this topic. Thus, it may represent one more resource in post-fracture treatment. Therefore, the aim of this study was to evaluate the effectiveness of Russian current in the consolidation of experimental tibial fracture in adult rats.

Methods

Experimental Design

In this experimental study, 24 Wistar rats (*Rattus norvegicus albinos*) were used, adult, male, weighing between 200 and 300 grams, maintained with photoperiod lamps in a 12-hour light/dark cycle and controlled room temperature of 25 degrees Celsius. The animals were fed standard feed and water *ad libitum*.

The animals were divided randomly into two groups: control group (CG), composed of 12 animals, and Intervention Group (IG) also composed of 12 animals, both animal groups underwent the osteotomy process. After the surgical procedure the IG was subjected to an electrical stimulation protocol with RC (average frequency), while the CG did not undergo any type of treatment.

Three animals from each group were euthanized at each evaluation moment in the following periods: 5th, 10th, 20th and 30th day of electrostimulation.

All ethical and legal requirements for animal research were met and all procedures of this study were approved by the Institutional Research and Ethics Committee (UNOESTE), protocol number: 009/2001.

Surgical Procedure

All the animals underwent the osteotomy procedure during which after 12 hours of fasting the animals were anesthetized with Thionembutal intraperitoneally at a dose of 45mg/kg. Next trichotomy of the left hindlimb was carried out and in the medial portion a longitudinal incision was made in the skin, of about 1.5 cm, with consequent exposure of the proximal medial aspect of the left tibia.

The musculature of the region was held back, and with the aid of a micro electric drill (RedLine -Bethil, São Paulo, Brazil), bit diameter 0.7 mm, a perforation was made, sufficient to reach the medullary canal of the tibia bone in the middle third of the diaphysis, without causing complete fracture of the tibia. No analgesics were administered and the animals were not restrained after surgery.

Electro-Stimulation Protocol

The electro-stimulation was initiated 24 hours after the osteotomy, being performed for 5 minutes once a day on each animal in the afternoon, using Endophasis-R equipment (model ET 9701, KLD Biossistemas Equipamentos EletrônicosLtda, Amparo, São Paulo, Brazil). To perform the protocol, TM-23 brass electrodes were prepared, 0.5 cm in diameter.

An average frequency current of 2500 Hz was used (Russian current), a modulation frequency of 85 Hz, and percentage of current in the cycle of 50%, with a contraction and rest time of 9 seconds, for a 5 minute period of application and intensity – motor level.

For each electro-stimulation session, the animals of the IG were anesthetized with Thionembutal intraperitoneally (45 mg/kg) for placement of the electrodes, specifically developed for this study, which were fixed in a standardized form immediately above and below the line of the surgical wound. After application of the electro-stimulation protocol and euthanasia of the animals with lethal doses of Thionembutal, the left tibia was removed for histological analysis (19).

Histological analysis

The bones were fixed in 10% formalin solution for 72 hours after which they were subjected to decalcification in a formic acid and formalin solution for three weeks. The pieces were washed in water, dehydrated, diaphanized, and embedded in paraffin. Subsequently, histological sections were obtained, seven micrometers thick, which were submitted to hematoxylin and eosin and Masson's trichrome staining for general analysis of morphology. The slides were analyzed using a standard optical microscope (Nikon, Labophot Model, Japan). Descriptive analysis of the results was performed.

Results

5th day of electro-stimulation: In the CG (without electro-stimulation) when euthanasia was performed six days after osteotomy (24 hours rest added to the 5 days of IG electro-stimulation), it was observed that in the periosteum, whose structure presented normal characteristics on most of the surface of the diaphysis, the portion covering the edges of the osteotomy orifice were thicker. However this presented a lower degree of primary ossification in the interior, where the presence of trabecular bone was almost imperceptible. In the deepest region of the wound orifice, corresponding to the region occupied by the endosteum, bone comparable to a callus was observed which formed a protrusion to the bone marrow. This structure occupied the majority of the medullary canal diameter and is formed by primary bone tissue, rich in trabeculars containing the normal elements of an ossification center, such as osteocytes and osteoblasts, as well as osteoclasts.

In the IG, in the period of the 5th day of electro-stimulation, the area of the diaphysis that was injured was covered by the periosteum, but with a thickness several times greater than that of normal rats. This tissue is particularly rich in cells and collagen fibers, containing in its interior numerous delicate trabeculae of primary bone. In the area corresponding to the orifice caused by osteotomy, the periosteum protruded into the interior, making contact with the endosteum.

In its structure many trabeculae of primary bone tissue were observed, being however thinner than the tissue overlying the edges of the orifice. At the level of the deep end of the hole, the endosteal presented intense osteogenic activity, organizing a primary bone tissue, which protruded toward the bone marrow.

The periosteum lining the edges of the orifice resulting from the osteotomy process revealed the presence of primary bone tissue organized in trabeculae, being in a more advanced stage of ossification than the tissue invading the osteotomy orifice.

In the same period, the tissues invading the osteotomy orifice were organized in a structure with a solid cylinder format. Its most superficial portion presented continuity with the periosteum that surrounds the edge of the orifice, and in the deepest end connected with primary bone tissue originated from the ossification process that occurs at the expense of activity of the endosteal that lines the diaphyseal channel. This tissue demonstrated a histological feature of primary tissue organization, being in a more advanced stage of ossification than that seen in the periosteum covering the osteotomy orifice.

In this tissue trabecular bone of different diameters and an anastomotic aspect were noted surrounding rounded cavities containing elements that comprised a differentiated connective tissue. Osteocytes were observed within the bone matrix and in the most outward position, a number of osteoblasts and osteoclasts.

It was also possible to observe that the periosteum revealed intense fibrotic activity. In its outer portion a large number of similar fibroblast cells were noted, containing abundant intercellular material formed mainly of collagen fibers.

In the innermost portion and contacting with the diaphyseal bone, this tissue contained many cells with a vacuolated appearance and varying rounded and elliptical shapes, separated by a wide matrix, this tissue is equivalent to the hyaline cartilage.

10th day of electro-stimulation: In the CG in this period, in the region corresponding to the edges of the osteotomy orifice, the periosteum appeared to be thick. Below this tissue, primary bone tissue was observed containing thick trabecular bone. In the portion in contact with the secondary bone tissue this tissue revealed more delicate trabeculae. In the deepest portion of the osteotomy orifice, corresponding to the endosteal, extended primary callus bone tissue was observed, filling the greater part of the medullary canal diameter. In some areas, this tissue was shown to be formed by thick trabecular bone.

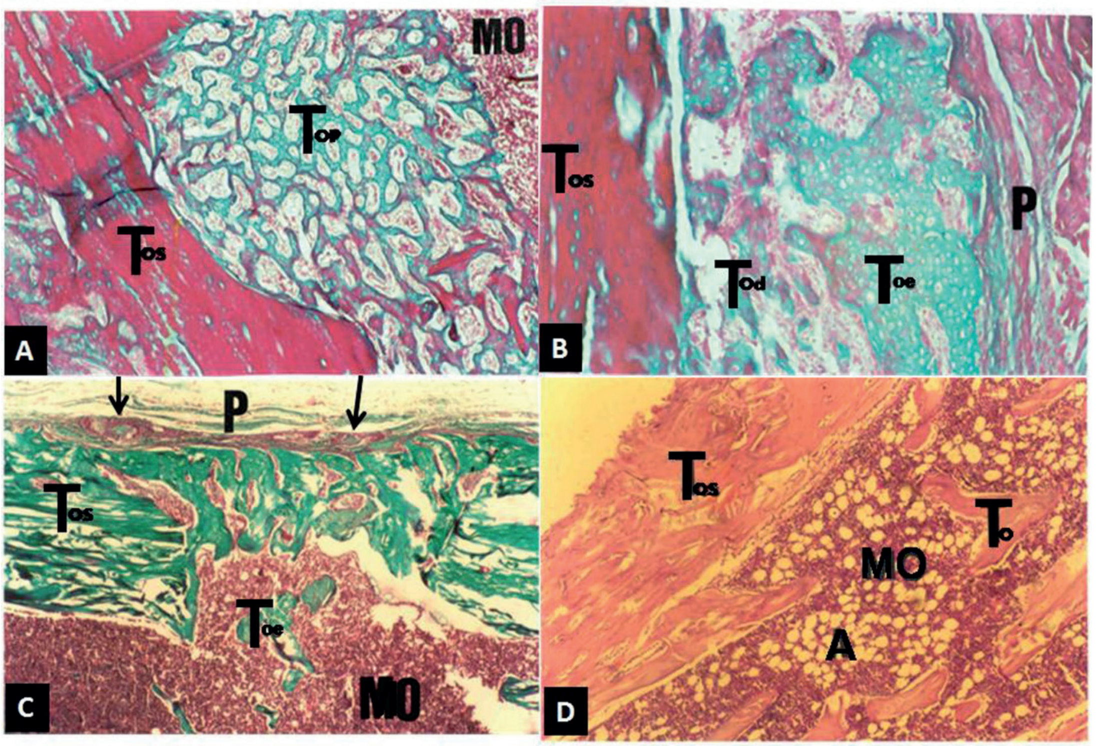

Figure 1 - Control Group. A. Longitudinal section of part of the diaphysis of rats after 5 days of osteotomy. The deepest portion of the osteotomy hole (cross-section) is filled with primary bone (Top), toward the bone marrow; secondary bone diaphyseal (Tos); Bone marrow (BM); Masson. 160X. B. Longitudinal section of rat after osteotomy (10 days). thick and fibrous periosteum (P); periosteal bone callus with thick trabeculae (Toe) and delicate (Tod); secondary bone diaphyseal (Tos). Masson. 40X C. Longitudinal section of diaphysis of rats after osteotomy (20 days). Periosteum (P) with trabeculae remains (arrow); secondary bone diaphyseal (Tos); trabeculae of endosteal bone (Tce); Bone marrow (BM). Masson. 20X. D. Diaphysis rat cut after osteotomy (30 days). secondary bone diaphyseal (Tos); bone trabeculae (To) within the bone marrow (MO), adipocytes (A). Hematoxylin and eosin. 20X.

Furthermore, associated with the endosteal callus, a fibrous tissue was observed, with dimensions equivalent to the diameter of the medullary canal.

In the IG in this period, the osteotomy orifice was observed filled by a connective tissue, inside which an active primary ossification process was observed. This tissue protruded toward the periosteum, forming a structure similar to bone callus, which in turn was covered by the normal periosteum.

In this phase, the tissue that filled the orifice, in a portion equivalent to half the depth of the orifice, presented characteristics of an active primary ossification process, being however in a less advanced stage than that observed in the bone callus. Surrounding this tissue, connective tissue was observed, containing delicate fibers with numerous cavity formations, of different diameters, being similar to blood vessels in formation.

In the deepest portion of the osteotomy orifice, corresponding with the endosteum, there was an expansion of primary bone tissue, also assuming the aspect of a callus protruding toward the medulla.

Furthermore, in this area bone tissue was observed, situated between the deepest portion of the orifice and bone marrow, which presented secondary bone tissue characteristics, being familiar to the diaphysis. This tissue reestablished the continuity of the diaphyseal bone.

During the replacement of connective tissue and cartilage tissue that comprise the structure which fills the osteotomy orifice, ossification begins in the periosteum, initially covering the edges of the orifice, protruding forward to the central portion of the orifice.

20th day of electro-stimulation: During this period in the CG, it was noted that the periosteum presented normal thickness and aspects, containing, however, few bone trabeculae in its interior. The majority of the bone callus of periosteal origin was shown to have been reabsorbed. The diaphyseal bone presented a continuous aspect in the most superficial portion and a discontinuous aspect, although containing some trabecular bone in its innermost portion.

In the IG, the osteotomy orifice appeared to be mostly filled by bone tissue with characteristics similar to diaphyseal tissue, and yet still with remains of primary bone and connective tissue. In some areas it was noted that the diaphyseal bone established continuity with the bone filling the osteotomy orifice.

Furthermore, the bone callus of endosteal origin had been mostly resorbed, reestablishing the continuity of the medullary canal. However, in the deepest portion of the osteotomy orifice the presence of fibrous connective tissue remains was still observed.

30th day of electro-stimulation: At this stage in the CG, secondary bone tissue replacement was observed, but with numerous gaps in the interior. In the endosteal region, we noted some bone formations still protruding toward the bone marrow. In addition, within the bone marrow, which contains numerous adipocytes, the presence of trabecular bone could be observed.

In the IG at this stage, the periosteum presented a normal structure. At the site of the osteotomy orifice, the diaphyseal bone continued in the superficial and deep portions, being less compact in the central region.

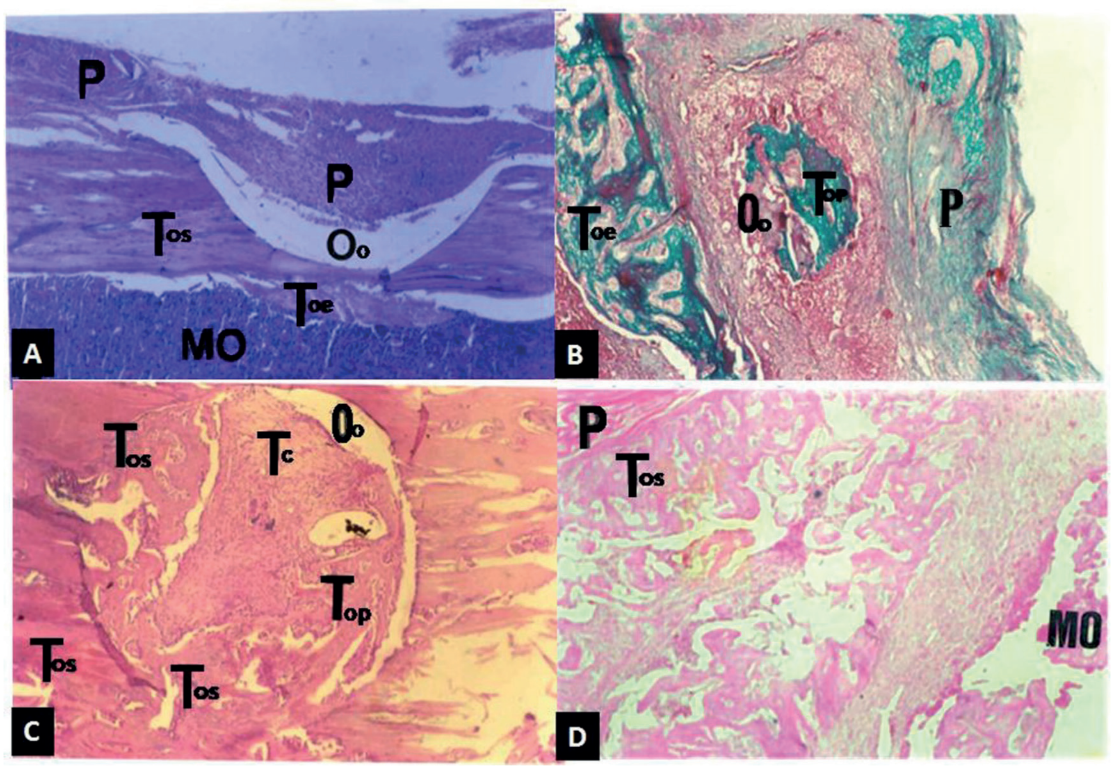

Figure 2 - Electrically stimulated group. A. Longitudinal section of middle third diaphysis of the rat subjected to osteotomy, followed by electrical stimulation (5 days). Periosteum (P); osteotomy hole (Oo); secondary bone (Tos); endosteal bone (toe); Bone marrow (MO). Hematoxylin and eosin. 20X. B. Longitudinal section of rat shaft subjected to osteotomy, followed by electrical stimulation (10 days). The hole is sectioned in the middle third. Orifice osteotomy (Oo); Primary bone tissue, similar to the periosteal callus, within the tissue that fills the hole (Top); periosteum (P); endosteal bone (Toe); Masson. 40X. C. Longitudinal section of rat shaft subjected to osteotomy followed by electrostimulation (20 days). In the center of figure, cross section of osteotomy hole (Oo) containing in its interior the secondary bone diaphysis (Tos), as well as primary bone remnants (Top) and connective tissue (Tc). Hematoxylin and eosin. 20X. D. Longitudinal section of diaphyseal, followed by electrical stimulation (30 days). Periosteum (P); secondary bone diaphyseal (Tos); Bone marrow (MO). Hematoxylin and eosin. 40X.

Discussion

In the present study we observed the process of bone regeneration by means of histological analysis of tibias of rats which underwent the osteotomy process, submitted to application of average frequency current at different moments of the repair process. The main results point to higher primary ossification, intense osteogenic activity, and increased periosteum thickness, characterizing more advanced ossification with a greater presence of trabecular bone marrow in the group of rats subjected to treatment with average frequency current.

The fracture repair process is a complex event involving several biological bone activities, such as remodeling, and intramembranous and endochondral ossification. Several studies have demonstrated, also by means of histological analysis, a higher periosteal reaction in animals under treatment with electrical stimulation in different modalities, compared to control groups (2, 7, 11, 18, 20). These results suggest that this type of intervention should be further explored (21, 22, 23).

The average frequency current presented positive results in the post-fracture intervention in rats. It is believed that this current has an action on bone morphogenetic protein, which stimulates tissue growth. It is suggested that a similarity exists between the mechanisms of action of ultrasound and RC. The electric current induces a cell mediation causing differentiation or modulation between the cellular reaction and electric charge, which can cause morphological and hormonal alterations such as an increase in the number of cells and secretory activity of the animal bladder. Thus it is suggested that the constant application of electrical potential to fractures may result in higher activity and stimulation of mesenchymal cells (2, 3, 5, 7, 22, 23).

The alterations caused in the bone tissue by applying electrical stimulation through Russian current and the vibrations triggered by the use of ultrasound can be attributed to the piezoelectric effect. Piezoelectricity is an effect that can be characterized by the property of certain materials to produce electric polarization by means of mechanical application, i.e., generate electric voltage through mechanical pressure or the reverse. Thus it is believed that this mechanism may be capable of stimulating bone growth and thus promoting the repair process (5, 7).

Furthermore, other effects of the application of this current have been described, such as increasing the mechanical properties of fracture callus by stimulating the synthesis of cartilage extracellular matrix proteins, thus altering the maturation of chondrocyte formation of endochondral bone; it is believed that a similar mechanism occurs in the application of RC (5, 7, 21, 22, 24, 25, 26).

It is known that RC can be used in several therapeutic aspects, such as global muscle strengthening, early intervention of postoperative muscle stimulation, and muscle stretching in hypertonic muscles (18). From the results of this study, one more use could be attributed to interventions with average frequency current as the qualitative analysis demonstrated promising results in the treatment of post-fracture rehabilitation. Thus the results presented in this study highlight the need for future studies that perform quantitative analysis on this subject, which may provide another resource in the treatment of fractures.

The following are presented as limitations of the study: the small sample size and the absence of quantitative analysis. Thus, we suggest that future studies be performed which encompass these issues and deepen the knowledge of the use of average frequency current in the bone repair process.

Conclusion

Thus, it can be concluded that the group electrostimulated by Russian current, with the parameters used in the present study, presented an accelerated osteogenesis process.

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