

Comparison of the Effects of Electrical Field Stimulation and Low-Level Laser Therapy on Bone Loss in Spinal Cord–Injured Rats

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Abstract

Objective: This study investigated the effects of low-level laser therapy (LLLT) and electrical stimulation (ES) on bone loss in spinal cord–injured rats. **Materials and Methods:** Thirty-seven male Wistar rats were divided into four groups: standard control group (CG); spinal cord–injured control (SC); spinal cord–injured treated with laser (SCL; GaAlAs, 830 nm, CW, 30mW/cm, 250 J/cm²); and spinal cord–injured treated with electrical field stimulation (SCE; 1.5 MHz, 1:4 duty cycles, 30 mW, 20 min). Biomechanical, densitometric, and morphometric analyses were performed. **Results:** SC rats showed a significant decrease in bone mass, biomechanical properties, and morphometric parameters (versus CG). SCE rats showed significantly higher values of inner diameter and internal and external areas of tibia diaphyses; and the SCL group showed a trend toward the same result (versus SC). No increase was found in either mechanical or densitometric parameters. **Conclusion:** We conclude that the mentioned treatments were able to initiate a positive bone-tissue response, maybe through stimulation of osteoblasts, which was able to determine the observed morphometric modifications. However, the evoked tissue response could not determine either biomechanical or densitometric modifications.

Keywords: *Spinal Cord Injury, bone loss, Electrical Field Stimulation*

Introduction

Less than 1% of people who sustain significant injury to the spinal cord fully recover their impaired neurologic functions, and a many such injuries result in partial or complete paralysis. The number of people in the United States who currently live with spinal cord injury (SCI) is estimated to be 253,000, with 11,000 new cases occurring each year. SCI is responsible for several physiological modifications, including a great decline in bone mineral density (BMD) and an increased risk of fractures. In this context, a critical need exists to develop technologies to treat bone loss after SCI. One promising treatment is the use of low-level laser therapy (LLLT), which seems to induce osteogenesis and stimulates fracture healing. Its action is based on absorption of light by tissues, generating, as a consequence, modifications to cellular metabolism. LLLT can modulate a series of cellular chemical reactions and stimulate mitochondrial respiration, molecular oxygen production, and ATP synthesis. In vitro studies using osteoblast cells showed that LLLT is capable of boosting mitochondrial activity, osteoblast DNA and RNA synthesis, bone nodule formation, osteocalcin and osteopontin gene expression, and ALP activity.

Similarly, a significant body of evidence has now been accumulated enabling researchers to expose evidence proving that electrical stimulation has a

positive effect on bone repair. Electrical stimuli of relative low amplitude and high frequency can influence in vitro and in vivo bone formation and resorption, suggesting that such a modality may be clinically used to inhibit or reverse osteopenia. According to Brighton et al., pulsed electromagnetic fields (PEMFs) promote cell proliferation caused by a Ca²⁺ ATPase trigger, which stimulates bone growing and repairing. Electrical stimulation also enhances membrane potential, resulting in conductivity increase and improved metabolism. Although the positive effects of LLLT and electrical stimulation on bone cell proliferation have been demonstrated, their effects on bone loss due to spinal cord injury are not well known. Before such therapies are reliably and broadly used as a therapeutic modality, it is necessary to investigate the effects and dose–response characteristics of such treatments in in vivo studies to determine their safety and efficacy. Consequently, we expected that laser therapy and EFS might accelerate bone metabolism of the femur and tibia in spinal cord–injured rats. In this context, our study investigated the effects of both treatments on bone loss in spinal cord–injured rats.

Material and Methods

Experimental design and surgical procedures

Thirty-seven male Wistar rats (aged 8 weeks and weighing 290_6.8 g) were used in this study. This study was approved by the São Paulo Federal University Animal Care and Use Committee guidelines (number 1617/08). Rats were randomly distributed into four groups: standard control group (CG), control without spinal cord-injured animals (n=7); spinal cord-injured control (SC), spinal cord-injured animals without any treatment (n=10); spinal cord-injured laser (SCL), spinal cord-injured animals treated with laser therapy (n=10); and spinal cord-injured electrical field (SCE), spinal cord-injured animals treated with electrical field (n=10).

Surgical procedure

The animals were anesthetized with an intraperitoneal (IP) injection of ketamine (90 mg/kg) and xylazine (10 mg/kg), and a laminectomy was performed at Th9-10. In injured rats, the dura mater was exposed, and the spinal cord was completely sectioned with microscissors. In sham-operated animals, the same laminectomy was performed, but the spinal cord was not sectioned. To reveal temporal changes in the locomotor function after SCI, an evaluation was carried out by using the Basso, Beattie, and Bresnahan (BBB) Locomotor Rating Scale.

Treatments

The treatments started immediately after surgery, and it were performed 3 times per week, during 4 weeks. A lowenergy GaAlAs (Teralaser, DMC São Carlos, SP, Brazil; 830 nm, CW, 0.028 cm² beam diameter, 100mW, 30mW/cm², 250 J/cm²) was used in this study. Irradiation was applied on both femora and tibiae (70 sec per point). The low intensity pulsed electrical field equipment (1.5 MHz, 1:4 duty cycles, 30mW, 20 min) was constructed in the electronics laboratory of the Bioengineering Department (University of São Paulo, São Carlos, SP, Brazil). Metal electrodes (25X35 cm) were positioned on the upper and lower parts of the cage to submit the entire body of the rats to a low-intensity electrical field through capacitated coupling, which does not cause any discomfort for the animals.

Analysis

Biomechanical, densitometric, and morphometric analyses were performed.

Results

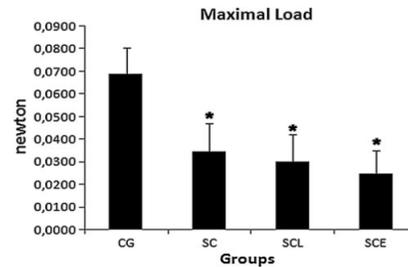
General findings

The lesion procedure caused severe degradation in behavioral performance, as measured by the BBB

score. Injured animals did not show any recovery in their general motor behavior, and none of them presented plantar placement of the paw with weight support during the experimental period.

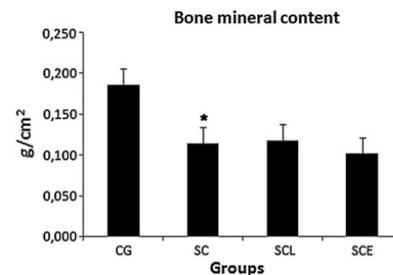
Biomechanical analysis

Statistical analysis showed that the CG maximal load, energy absorption, and structural stiffness were higher than the values found in the other groups. No other difference was found between the rats without treatment and the treated injured ones.



Densitometry

Statistical analysis revealed lower BMD and BMC of the SC group when compared with the CG. No other difference was observed.



Morphometric analysis

Spinal cord-injured rats with no treatment showed a statistically significant difference when compared with the CG and spinal cord-injured animals treated with an electrical field regarding the following variables: inner diameter, internal area, and external area of tibia diaphysis. The animals exposed to laser irradiation manifested a minor, though consistent, trend toward increased inner diameter, internal, and external areas, when compared with spinal cord-injured animals. However, the differences were not statistically significant between SCE and CG.

Discussion

Because previous in vitro and in vivo studies demonstrated the high osteogenic potential of low-level laser therapy and electrical stimulation, we hypothesized that both treatments might be used to

stimulate bone tissue and to avoid bone loss after spinal cord injury. Results from the present work showed that, in histologic analysis, both treatments triggered a minor increase in morphometric parameters and suggested that LLLT and the electrical field might have some stimulatory effects on bone metabolism in SCI rats. SCE rats showed significantly higher values of inner diameter, internal, and external areas of tibia diaphysis, and the SCL group showed a trend toward the same result (versus SC). Yet, such treatments were not able to improve either bone mineral density or biomechanical properties of spinal cord-injured rats. The osteogenic effects of laser therapy on osteoblastic cell proliferation and bone metabolism are well known. Kazem Shakouri et al. (unpublished data) also demonstrated that the use of laser boosted callus development in the early stage of the healing process in rabbits, with doubtful improvement in biomechanical properties of the healing bone. Moreover, LLLT has been proven to stimulate bone metabolism in osteoporotic rats and to accelerate fracture bone healing in osteoporotic rats. Similarly, studies using low-intensity electrical stimulation have manifested stimulatory effects on bone in many animal models, such as male rats with disuse-induced osteoporosis after limb immobilization, after sciatic neurectomy, and after ovariectomy. Pulsed electromagnetic fields can significantly suppress trabecular bone loss and restore trabecular bone structure in bilaterally ovariectomized rats evaluated through histomorphometry. Our results demonstrated that this experimental model produced a significant decrease in bone density and biomechanical properties in injured rats' femurs and tibiae. It is well known that spinal cord injury is followed by severe bone loss, mainly in the lower extremities and pelvis of patients and animals. That experimental model may be the most rapid of various types of osteoporosis. BMD reduction, deteriorated microarchitecture, and high fracture risk have been documented in SCI. We can hypothesize that the intense bone loss after SCI may partially explain the lack of LLLT and electrical-field effects on bone mass and on biomechanical properties in the treated animals in this study. Conversely, morphometric analysis showed some stimulatory effects of the treatments, mainly on the electrical field-treated rats, even after just 12 sections of treatment. It is possible to suggest that the LLLT and electrical field were able to initiate a positive bone-tissue response, maybe through the stimulation of osteoblasts, which might determine the observed histologic modifications. However, the evoked tissue response was not able to determine biomechanical

or densitometric modifications. We considered the methods used in this study very adequate to investigate the effects of LLLT and electrical field on bone loss after SCI. The three-point bending test and densitometry are widely used to measure the action of treatments on bone mechanical strength and bone-mineral density, respectively, in several bone disorders.

Conclusions

In summary, such findings suggest that although LLLT and the electrical field presented a positive effect on histologic parameters in SCI rats, no modifications to biomechanical and densitometric analysis were found, at that stage. It seems that the used treatments represent perhaps some of the most promising treatment modalities capable of stimulating bone tissue in different conditions by incrementing osteoblast adhesion and vessel migration. Considering this, further long-term studies should be developed to provide additional information concerning the late stages of bone-matrix synthesis and degradation induced by LLLT and electrical field on the bone tissue of spinal cord-injured rats. Such additional investigations should focus on the final goal of induced bone regeneration: restoring bone architecture to biologic and mechanical properties similar to those of an uninjured bone.

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