

EFFECT OF LOW-LEVEL LASER (GAAS, 904 NM) FOR BONE REPAIR ON FRACTURES IN RATS

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ABSTRACT

Objective: To analyze the effects of low-level laser therapy (GaAs, 904 nm) for bone repair on tibial fractures in rats. **Methods:** Forty rats were divided into four groups of 10 animals: control group without fracture (CG); fracture group without treatment (EG II); fracture group treated with laser at 10 J/cm² (EG III); and fracture group treated with laser at 15 J/cm² (EG IV). The fracture was produced surgically and the treatment lasted 45 days, done on alternate days. After treatment completion, the rats were sacrificed. The tibiae were radiographed and subjected to mechanical three-point flexion tests in order to evaluate the maximum force (N) required to break them. **Results:** The observed maximum force values (N)

were: control group (CG) of 51.5 N ± 7.9 N; EG II 17.2 N ± 7.8 N; EG III 16.6 N ± 12.1 N; and EG IV 30.3 N ± 7.8 N. There were statistically significant differences between the control group and the experimental groups and also between experimental group IV and the other experimental groups (II and III). Radiographs showed callus formation in all the fractured groups, thus indicating that they had undergone the normal tissue repair process. **Conclusion:** EG IV, which underwent laser therapy with a dosage of 15 J/cm², showed the highest maximum force value (N) among the experimental groups, thus demonstrating the influence of higher laser dosage on bone repair.

Keywords – Lasers; Fractures, Bone; Fracture Healing

INTRODUCTION

Fractures may be caused by diseases, trauma or surgical procedures, through direct or indirect mechanisms. In direct mechanisms, the bone is hit by, or it hits a hard object or surface, thus producing a fracture at the same site as the trauma occurrence. In indirect mechanisms, there is a torsional or angular force that results in a fracture at the site where the tensions accumulate or where the bone is most fragile⁽¹⁾. Given the high incidence of fractures consequent to car accidents, falls and sports trauma, the bone repair process has been a topic studied worldwide⁽²⁾.

Bone consolidation does not always take place in the expected manner: it can become irregular, with delayed consolidation, and this may lead to the appearance of pseudarthrosis⁽³⁾. In this regard, different therapeutic resources have been used with the aim of accelerating the repair process and im-

proving the quality of bone consolidation⁽⁴⁾. Thus, studies have been conducted to evaluate the influence of application of electric currents, ultrasound and laser on the bone repair process⁽⁵⁾.

In view of the points raised above, the present study had the objective of analyzing the effects of gallium arsenide low-level laser radiation (GaAs; 904 nm) for repairing tibial bone fractures in Wistar rats, by means of mechanical tests and radiological examinations.

MATERIALS AND METHODS

The project for this study was approved by the Ethics Committee for Animal Experimentation of the University of Uberaba (UNIUBE), at a meeting on September 17, 2009 (CEEA Official Letter 141/2009).

For this study, 40 Wistar rats were used. Their mean body weight was 247.1 ± 32.87 grams and their mean age was 45 days.

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The animals were divided into four experimental groups containing 10 animals each: control group (CG), without any fracture; experimental group II (EG II): the animals were subjected to fracturing of the right tibia by surgical means and did not receive any treatment up to the day of sacrificing; experimental group III (EG III): the animals were subjected to fracturing of the right tibia by surgical means and were treated with GaAs laser (gallium arsenide; 904 nm) at a dose of 10 J/cm²; experimental group IV (EG IV): the animals were subjected to fracturing of the right tibia by surgical means and were treated with GaAs laser (gallium arsenide; 904 nm) at a dose of 15 J/cm².

Before the fracturing procedure was performed, the animals were anesthetized with thiopental sodium at a dose of 0.1 mg for each 100 grams of the animal's body mass and the anterior region of the lower leg was shaved. Asepsis was performed at the site using 1% polyvinylpyrrolidone-iodine. The animals received antibiotic therapy consisting of applications of ampicillin (0.02 mg/100 g of the animal), intramuscularly, every 12 hours, starting one day before the fracturing procedure was performed, and continuing for seven days after the procedure.

To reach the tibia, a surgical incision of approximate length 1 cm was made in the anterolateral region. The fracture was started in the region of the proximal to middle third of the tibia by drilling, and it was completed by using a scalpel blade to "saw" the tibia to divide it into two pieces.

After the fracture had been produced, the tibia was stabilized using a 30 mm x 0.8 mm needle (Figure 1), which was used as an intramedullary nail. Starting on the day on which the fractures were produced, the animals received applications of Ketofen® (0.02 mg/100 g of the animal), intramuscularly, every 24 hours for three days, which was used as an anti-inflammatory agent and analgesic.

The animals in the two experimental groups (III and IV) were subjected to 22 sessions of applications of gallium arsenide laser (GaAs; 904 nm), using the Laserpulse Special Diamond apparatus (IBRA-LC904), at a power of 30 mV, on alternate days. Point applications of laser irradiation were made at three sites on the anterior face of the lower leg over the focus of the fracture: one point at the fracture site, another 1 cm above it and the third 1 cm below it (Figure 2).

For radiological control and evaluation of the evolution of the bone repair, radiographs were produced



Figure 1 – Tibia stabilized with needle used as an intramedullary nail. Source: University of Uberaba.



Figure 2 – Application of gallium arsenide laser (GaAs) to the right paw of the animal. Source: University of Uberaba.

just after the fracturing and on the 15th, 30th and 45th days after this, using the Dabi Atlante Spectro 70X dentistry equipment in lateral view, with film placed below the lateral region of the tibia, and with a collimator-leg distance of 8 cm. To produce the radiographs, the animals were anesthetized with an association of 0.2 mg/100 g of xylazine hydrochloride and 0.2 mg/100 g of ketamine hydrochloride, intramuscularly.

On the 46th day, the animals were sacrificed using thiopental sodium at a dose of 2 mg for each 100 g of the animal's body weight. The tibias were then dissected, the needles that had simulated intramedullary nails were removed and the bones were frozen at -27°C for the subsequent procedure of mechanical testing. For this, the bones were defrosted over a 24-hour period, while kept in an ordinary refrigerator, and were maintained in a saline solution until the time of the test, as described by Pessan et al⁽⁶⁾.

The bones were positioned in a holder for the destructive mechanical flexion test at three points to be carried out, such that the force imposed on the bone was applied perpendicularly to the focus of the fracture.

For this trial, a universal testing machine (EMIC® model DL-3000) belonging to the Dental Materials Research Laboratory of the University of Uberaba was used. It was equipped with a Kratos® load cell of capacity 50 kgf. The machine was connected to a microcomputer equipped with software capable of registering the load and deformation values during the tests. Among the test parameters, a drop velocity of 0.2 mm/min was standardized. The force applied and the displacement were monitored and recorded by means of specific software for the equipment, which also supplied the force-deformation curve for each test (Figure 3). The resultant maximum force value (N) was recorded, and this was the parameter used for the present study.



Figure 3 – Fractured tibia undergoing three-point mechanical flexion test. Source: University of Uberaba.

RESULTS

Figures 4, 5 and 6 show radiographic images of the right tibias, produced shortly after the surgery to create the fracture (A), and on the 15th (B), 30th (C) and 45th (D) days.

The radiographs produced on the 15th, 30th and 45th days demonstrated that bone callus was forming around the region of the fracture, in all the groups studied, with a gradual increase. Thus, it can be suggested that the bone consolidation process, which is preceded by formation of a fibrous callus, had begun. However, from the present analysis, it was not possible to qualify and quantify the bone repair process by comparing the different groups. Figure 7 presents the resultant means and respective standard deviations for the maximum force values (N) obtained in the mechanical flexion tests at three points for each group studied.

It could be seen from the analysis that there were statistically significant differences in comparing the mean maximum force values (N) between the experimental groups and the control group, which showed that effective fractures had been produced. Even with the laser stimulation, the values obtained in experimental groups III and IV, in comparison with the CG, indicated that the bones had not returned to the resistance level found in the bones that had not undergone fracturing.

There were statistically significant differences between the experimental groups (EG II, EG III and EG IV), and the mean maximum force values (N) of experimental group IV were highest.

DISCUSSION

To carry out this study, Wistar rats were chosen as the experimental animals because they are easy to handle, have a low cost, need less space and can

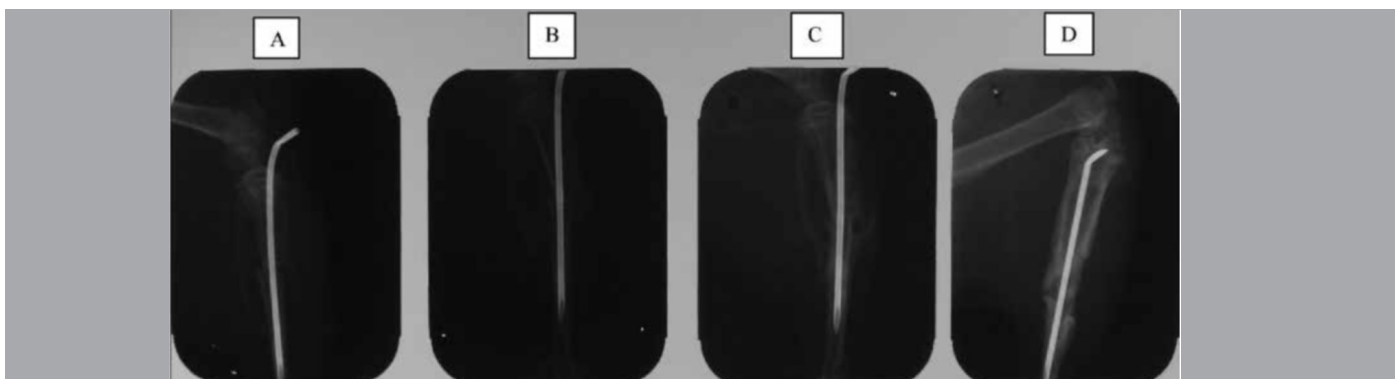


Figure 4 – Lateral-view radiographs on experimental group II (EG II): (A) just after fracturing; (B) on 15th day; (C) on 30th day; and (D) on 45th day. Source: University of Uberaba.

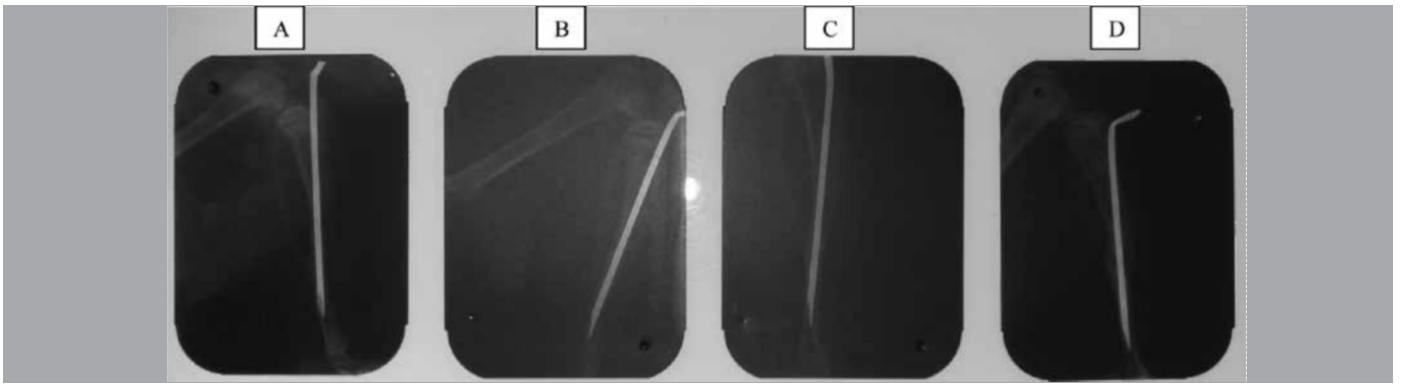


Figure 5 – Lateral-view radiographs on experimental group III (EG III): (A) just after fracturing; (B) on 15th day; (C) on 30th day; and (D) on 45th day. Source: University of Uberaba.

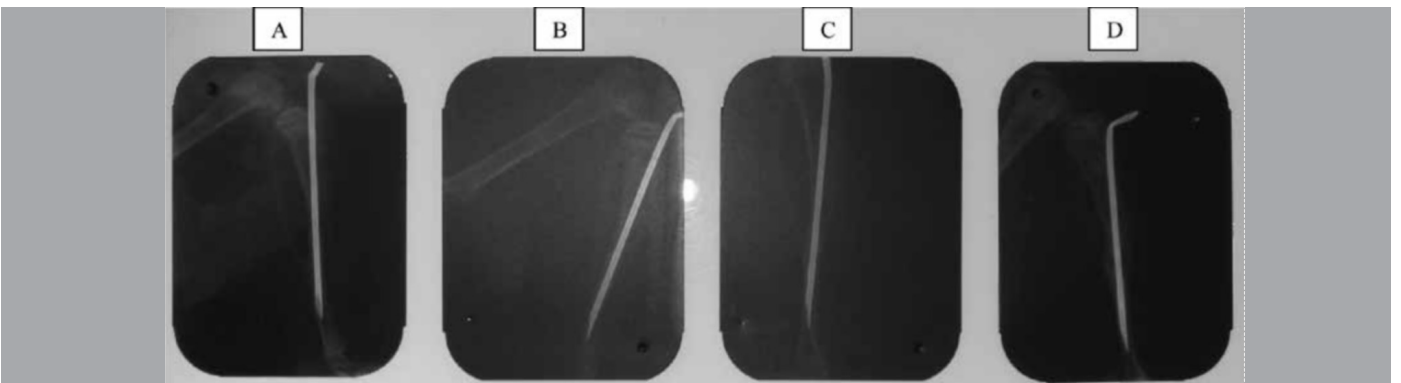


Figure 6 – Lateral-view radiographs on experimental group IV (EG IV): (A) just after fracturing; (B) on 15th day; (C) on 30th day; and (D) on 45th day. Source: University of Uberaba.

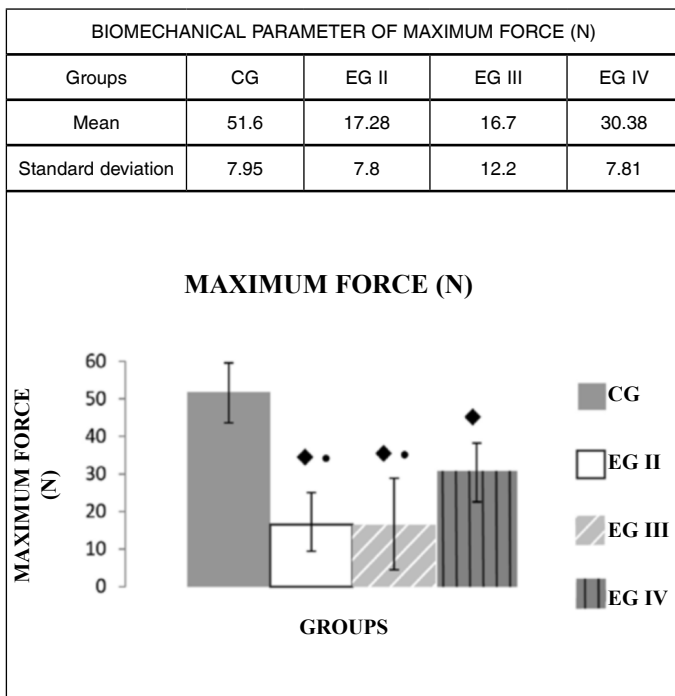


Figure 7 – Comparison of mean maximum force (N) between the groups. The symbol ♦ represents statistically significant differences shown by the experimental groups II, III and IV (EG II, EG III and EG IV) versus the control group (CG); the symbol • represents statistically significant differences shown by experimental group II (EG II) and experimental group III (EG III) versus experimental group IV (EG IV).

easily be obtained from the vivarium of the University of Uberaba. Male rates were chosen in order to avoid the bone alteration caused by female sexual hormones, thereby maintaining a control pattern for increases in body weight, and for linear growth and growth in thickness⁽⁷⁾.

Because bones, together with muscles, are responsible for support, movement and protection of vital organs, they are constantly subjected to traction, compression, torsion and flexion forces. They generally bear a combination of these forces, which makes it useful and necessary to know about their mechanical properties in order to assess their integrity⁽⁸⁾.

Since the time of Galileo Galilei’s observations, it has been assumed that bone architecture is influenced by the mechanical tension associated with its function. From the 19th century onwards, this relationship between structure and function became known as Wolff’s law. The principle of this law is based on the concept that there is a correlation between the trabecular alignment patterns and the directions of the tensions that occur through functioning⁽⁶⁾.

It is known that density and mechanical strength measurements are important characteristics in evaluating

bone tissue. The guidelines proposed by the FDA (Food and Drug Administration) suggest that measurements should be made not only on bone density and biochemical markers of bone turnover, but also on bone strength through biomechanical tests. Such tests have been reported by various authors to be one of the ways of assessing bone consolidation⁽⁹⁾.

During the three-point mechanical flexion test, the deformation over which force and displacement are proportion produces a linear graphical relationship known as the elastic phase of deformation. This deformation is not permanent, which means that when the applied load is removed, the material will return to its original shape and remain undamaged. If the force continues to be applied (maximum force), permanent deformation occurs (plastic phase). From an atomic perspective, this corresponds to breakage of bonds between neighboring trabeculae. Even if the force is then removed, the material will not return to its original position, thus leading to structural lesions and consequently, bone fractures⁽¹⁰⁾.

In the present study, the three-point mechanical flexion test was sufficient for the proposed analysis, since it is an easily applied and widely used test within scientific settings.

Several studies have reinforced the success of low-level laser therapy for treating fractures⁽¹¹⁻¹⁷⁾. However, despite the positive results found, these studies have used laser radiation emission devices with different wavelengths and very different doses and treatment durations, thus making it difficult to compare them and standardize a treatment protocol. The doses of 10 J/cm² and 15 J/cm² used here were chosen based on studies found in the literature, and these are the values most encountered, or close to those most encountered^(13,15,17).

The total treatment duration was set as 45 days, on alternate days, because this is the mean time needed for consolidation of the type of tibial fracture produced in this study⁽¹⁸⁾ and because a pilot study conducted previously using different lengths of treatment showed that the best quality of bone consolidation was on the 45th day.

The biomechanical evaluation was conducted by comparing the parameter of maximum force (N), and this showed that laser radiation treatment at a dose of 15 J/cm² for the length of time studied had positive effects regarding fracture repair. It facilitated formation

of a fibrous callus and consequently, bone tissue repair. These results confirm those of Liriani⁽¹⁶⁾, who found a greater maximum force value (N) in the group of animals that received fractures and were treated with laser radiation than in the non-irradiated group.

On the other hand, laser radiation with a dose of 10 J/cm²⁽⁹⁾ was not shown to be effective in accelerating the bone repair process in tibial fractures in rats, since the maximum force values (N) obtained in the three-point mechanical flexion test were not statistically different from the values obtained in the EG II group.

Pinheiro et al⁽¹²⁾ found positive effects regarding bone realignment after infrared laser irradiation (AlGaAs; 830 nm) and suggested, on the basis of previous studies⁽¹⁹⁾, that undifferentiated mesenchymal cells were positively biostimulated to become osteoblasts, which more rapidly became osteocytes. The biological mechanisms involved in the improvement of bone tissue growth through low-level laser irradiation are still not clearly understood. The hypothesis advocated by Freitas⁽²⁰⁾, and corroborated by other studies, is that the laser energy may excite porphyrins and cytochromes (which are intracellular chromophores) and thus induce an increase in cell activity and consequently an increase in ATP concentration, which releases calcium. These morphological changes that occur in the bone structure after exposure to laser radiation may explain the increase in maximum force (N) in the group treated with 15 J/cm², given that through facilitation of bone realignment, greater numbers of biostimulated cells in osteocytes and increased ATP and calcium concentrations, the bones probably were able to bear a greater load imposed on their structure during the three-point mechanical flexion test.

Through the 15-day intervals between radiographs used in this study, the evolution of the bone consolidation process of the fractures could be analyzed, thus simulating clinical practice for assessing bone repair in fracture cases.

The radiographic analysis demonstrated that bone callus formed in all the fractured bones, independent of whether the group was irradiated or not. However, this analysis was insufficient to quantify the bone repair process by comparing the different groups, given that all the groups presented bone callus formation in a similar manner.

It is suggested that new studies should be conducted to analyze other mechanical properties, such as

deflection (mm) and stiffness (N/m) of the bone, along with analysis on bone density, which may complement the results encountered in the present study.

Lastly, we believe that there is a need for further studies on the effects of low-level laser therapy on the whole process of bone repair, especially analyzing the effect of the laser irradiation at cell and biomolecular levels, with the aim of establishing an effective protocol for treating bone fractures, based on scientifically proven parameters.

CONCLUSION

It can be concluded from this study that low-level laser therapy showed positive results in the bone re-

pair process on tibial fractures in rats, thereby increasing the maximum force withstood by the group treated with a dose of 15 J/cm².

It was also observed that the dose of 10 J/cm² was not effective in accelerating the bone repair process in fractures, thus showing that bone tissue repair is dose-dependent, and that higher doses seem to be more effective for stimulating the bone repair process.

New methodology was established for carrying out the surgery, with the aim of obtaining fractures for conducting studies on bone lesions.

The three-point mechanical flexion test was shown to be efficient for analyzing the mechanical property studied here (maximum force for breakage), in the control and experimental groups.

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