Ultrasound in Speeding up the Fracture Healing Process in Rats

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Abstract. In different previous studies it has been reported that ultrasound shortens the healing period of fresh fractures (Leung et al., 2004) and promotes the healing of refractory fractures (pseudoarthrosis, delayed union fractures), shortening the treatment period (Miyabe et al., 2010).

The aim of our study was to test various parameters of ultrasound and determine the optimum parameters for the fracture healing process hastening in recent fractures. For this we surgically induced fracture of the radius bone from the right thoracic limb to a total of 20 rats. Following surgery, they were divided into 4 groups to receive different treatment as follows: a control group that received no treatment, and 3 groups that received different doses of ultrasound, namely: intensity of 0.1 W/cm² in pulse mode, intensity of 0.5 W/cm² in pulse mode and intensity of 0.5 W/cm² continuously. Treatment began in the 7th day after fracture induction and was applied for 10 days as follow: 5 days treatment, two days off, five days treatment.

At 21 days after surgery, the animals were euthanized to harvest samples. In interpreting results macroscopic and histopathological examination were performed. On slaughter, all treatment groups, regardless of the applied dose, had more advanced stages of callus formation compared with controls, and between treatment groups, the most advanced bone healing process was observed in the group treated with ultrasound with intensity of 0.5 W/cm² in pulsed mode.

Keywords: ultrasound therapy, fracture healing, recovery.

INTRODUCTION

Ultrasound has been shown to accelerate fracture healing in both animal models and clinical trials, but the mechanism of action remains unclear (Azuma et al., 2001). Another question that still has no clear answer refers to the dose applied, in this respect being many controversial opinions. The frequency used ranges from 0.8 to 15.0 MHz, which is based on considerations of sound absorption, penetration, and resolution. The intensities for therapeutic applications are high (1–50 W/cm²) and are designed to cause significant tissue heating (Azuma et al., 2001).

In particular, it has been reported that low intensity pulsed ultrasound shortens the healing period of fresh fractures (Leung et al., 2004) and promotes the healing of refractory fractures such as pseudoarthrosis and delayed union fractures, shortening the treatment period (Miyabe et al., 2010).

The positive effect of ultrasounds on fracture healing may be caused by a stimulation of the different cellular processes involved in fracture repair and bone formation, such as
angiogenesis, chondrogenesis, and intramembranous and endochondral ossification (Rutten et al., 2008).

The aim of this study was to test different doses of ultrasound and determining their effectiveness, to implement in current practice most appropriate dose in speeding up the fracture healing process in pets.

MATERIALS AND METHODS

The study has been carried out on the Surgery Clinic of Veterinary Medicine Faculty from Cluj-Napoca, during May-June 2012, on a number of 20 laboratory rats from wistar line, aged 6 months, all males and having a weight of about 300-400g.

Research included, sequentially, several steps, as follows: animals’ acclimatization, fracture induction, the treatment and samples harvesting and processing.

Before the actual experiment, animals were brought in our biobase, where they were housed in individual cages and received the same fluid and food treatment. Acclimatization aims crossing the peak of adaptive responses to new environmental conditions and improve the general condition of animals, which is clearly affected by the contact with the new environmental conditions.

Experiment was required to achieve surgical induction of radius fracture from right thoracic limb in all animals. Surgery was performed under neuroleptanalgesia protection, this being accomplished with Acepromazine (0.2 ml or 2 mg/animal, IM) and Ketamine (0.2 ml or 10 mg/animal, IM).

The surgery itself assumed to follow the following steps: skin incision (fig. 1) on the anterior part of the forearm region for an easier approach of radius; regional muscle dilacerations using a forceps (fig. 2) to reveal the 2-ray bone: radius and ulna; removal of tissue (fig. 3) to highlight the best possible visibility of radius bone; radius bone fracture performance using a clamp (fig. 4) and excision of a portion of about 1 mm of bone length; anatomical plans restoration by applying the suture points. To prevent infection, general antibiotic therapy was used, Enroxil, 0.1 ml / animal, administered im, single dose, and local with antibiotic spray. To check the success of surgery and bone fractured position we have performed a radiological examination postoperatively (fig. 5).

After fracture induction, the animals were kept at rest for 7 days. At 7 days after surgery, animals were divided into groups, as follow: a control group, witch received no treatment, and 3 groups witch received different doses of ultrasound (group 0,1i, received ultrasound with intensity of 0.1 W/cm² and frequency 0.5 MHz, in pulsed mode, group 0,5i received ultrasound with intensity of 0.5 W/cm² and frequency 0.5 MHz, in pulsed mode, and group 0,5c received ultrasound with intensity of 0.5 W/cm² and frequency 1 MHz, in continuous mode. Differential treatment was applied using an ultrasonic device Misonic 12 (fig. 6).
Fig. 1. Skin incision on the anterior part of the forearm region.

Fig. 2. Regional muscle dilacerations.

Fig. 3. Tissue removal for a better approach.

Fig. 4. Radius bone fracture performance.

Fig. 5. Postoperative radiological examination.

Fig. 6. Ultrasound therapy unit.
RESULTS AND DISCUSSIONS

To check the effectiveness of the applied ultrasound doses, were performed macroscopic and histopathologic examination of the formed bone callus. For this, animals were sacrificed at 21 days after fracture.

Although is a guidance exam, gross examination (fig. 7) provided valuable data. In the control group we could observe a poorly developed callus, with low resistance to tensile and torsional, and beam diameter not exceeding the bone. Its characteristics guide to an early stage, a fibro-cartilaginous callus. All of treatment groups showed a more advanced callus, well developed, with a larger diameter than the diameter of the radius bone, and with increased resistance. Macroscopic differences between treatment groups were quite small, yet they exist. These were only in the callus diameter size.

Fig. Macroscopic aspects of the fractured radius, postmortem, at 21 days after fracture:

a). Radius and ulna (group C): callus poorly developed, low resistance to tensile and torsional, beam diameter not exceeding the bone;
b). Radius and ulna (group 0.1i) callus in advanced development phase, durable, featuring entirely broken ends;
c). Radius and ulna (group 0.5i): advanced callus, highly resistant, with a diameter larger than the diameter of the radius bone;
d). Radius and ulna (group 0.5 c): callus in the most advanced stage of development, with the largest diameter, highly resistant to tension and torsion.
The exam that made the difference was the histopathology. On histopathological examination were revealed different stages of callus existing in different groups. Compared with controls, treated groups, regardless of the used parameters, had more advanced stages of the callus formation process. Thus, in the control group, histological examination revealed at the fracture site a callus mainly fibrous, with cartilaginous metaplasia of low intensity. In the group 0.1i, on histopathology could be seen a fibro-cartilaginous callus, with rare areas of bone metaplasia, and the presence of a cartilaginous bridge between the radius and ulna. In the 0.5i group we were able to observe a more advanced stage of callus formation, with intense bone metaplasia (center) and fibrous tissue in very small amount. The most advanced stage of bone callus formation could be observed in the batch 0.5 c, confirming data observed macroscopically. In this lot we have seen affront fractured ends with an intense bone metaplasia cartilaginous tissue, as confirmed by the presence of large amount of young trabecular bone (fig. 8).

In a survey conducted by G. Ter Haar in 1985 showed that there were wide variations in the use of ultrasound, including a range of intensities from 0.1 to 3.0 W/cm² showing a variation factor of 30 from lowest to highest applied intensity (T Watson, 2008). Beneficial effects were observed using both high doses, about 500 mW/cm² (Chang et al, 2002), but also with small doses of 30 mW/cm² (Azuma et al., 2001).

Another question mark is placed by the application and frequency of use. In this regard were studied various variations seen in the ultrasound effects on fracture healing at the same intensity but different frequencies (Tsai et al, 1992).
CONCLUSION

In a study, using different parameters of ultrasound to speed recent fracture healing, we extract some important conclusions, namely:

- Regardless of the parameters used, ultrasound proved to be beneficial in larger or smaller proportions in speeding recent fracture healing.
- Most effective in speeding healing fractures had the ultrasound treatment with intensity of 0.5 W/cm² and frequency 1 MHz, in continuous mode. This treatment led to the formation of an advanced bone callus confirmed by the presence of intense bone metaplasia.
- Ultrasound proved to be a very beneficial adjunct treatment in speeding the bone callus formation in recent fractures, easy to apply with no side effects, reasons to justify their introduction into clinical practice.

REFERENCES


