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Research Paper
Bone Biology

Effects of low-intensity pulsed ultrasound on healing of mandibular bone defects: an experimental study in rabbits

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Abstract. Research evidence suggests that low-intensity pulsed ultrasound (LIPU) produces significant osteoinductive effects, accelerating the healing of bone defects. The authors investigated the effects of LIPU on mandibular bone defects in a rabbit model. Fifty-six adult Dutch rabbits were divided randomly into control, LIPU-1 (1 MHz), and LIPU-3 (3 MHz) groups. A mandibular defect was created in all rabbits. The effect of LIPU on mandibular defects was assessed by frequency (1 or 3 MHz) and timing (2 and 4 weeks). Bone mineral density (BMD) was measured and stereology and histology performed; results were compared at the end of 2 and 4 weeks. LIPU-3 resulted in significantly higher bone formation compared to the control group at the end of week 4 on histological assessment ($P = 0.008$). BMD was significantly higher at 4 weeks than at 2 weeks ($P = 0.03$). LIPU-3 increased the numerical density of osteoblasts and osteocytes at the end of week 4 ($P = 0.05$ and $P = 0.001$, respectively). The results of this study are in favour of using LIPU 3 MHz to accelerate mandibular bone healing. However, this study suggests that a frequency of 3 MHz and the longer application of LIPU 3 MHz for 4 weeks can only promote 8% mandibular bone healing in rabbits. Therefore, the use of LIPU has no really convincing, consistent clinical effects on maxillofacial bone healing.

Key words: LIPU; healing; mandible; histology; stereology; rabbit.

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The healing of bone defects in the maxillofacial region is always a challenge in cases of congenital or traumatic defects or tumour resection.[1] Rapid soft tissue growth inside the defect may block bone formation at the defect periphery, resulting in impaired defect healing.[2] A relative lack of certain tissue factors in the centre of the defect, which originate from the edge of the defect, is believed to limit the bone healing process.[2]

Several methods for managing bone defects have been introduced following research in the field of maxillofacial bone regeneration, such as the use of bone grafts and/or barrier membranes.[3] Recently, the

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Table 1. Overview of previously reported studies on the application of low-intensity pulsed ultrasound (LIPU) on mandibular defects.

Reference	Species (*n*)	Frequency, MHz	Follow-up, weeks	Evaluation methods	Results
El-Bialy et al., 2002[7]	Rabbit (21)	1.5	4	Bone photodensity, vibratory coherence, mechanical stiffness, histological studies	Accelerated bone formation evaluated by photodensitometric, vibratory, elastic, and histological techniques
Schortinghuis et al., 2004[20]	Rat (72)	1.5	2, 4	Microradiographs	No statistically significant difference in the percentage of defect closure between the groups
Schortinghuis et al., 2005[6]	Rat (64)	1.5	2, 4	Microradiographs	No statistically significant difference in the percentage of defect closure between the groups
Erdogan et al., 2006[9]	Rabbit (30)	1.5	3	Three-point bending test, digital radiodensitometric analysis, histological and histomorphometric examinations	Considerable contributions to bone healing in mandibular fractures
El-Bialy et al., 2008[8]	Rabbit (36)	1.5	1, 2, 3, 4	Quantitative bone density (QBD), mechanical testing, and histological examination	Earlier stages of bone healing were enhanced more by continuous ultrasound, whereas late stages were enhanced more by pulsed ultrasound

effect of ultrasound on bone defect healing has been assessed. Well-designed prospective studies have been reported, indicating that low-intensity pulsed ultrasound (LIPU) can accelerate fracture healing of the long bones in animal models and the fracture repair process in the tibia and radius.[4]

Reports on the application of LIPU in the maxillofacial area are conflicting (Table 1). Ustun et al. reported that LIPU may have positive effects on osseointegration and the stability of dental implants.[5] However, Schortinghuis et al., in two separate studies, reported that there was no statistically significant difference in the percentage of defect closure between groups of rats exposed to LIPU and control rats.[1,6] El-Bialy et al., in two different studies, showed that LIPU 1.5 MHz accelerated bone formation in mandibular bone distraction and suggested that the later stages of healing were enhanced more by pulsed ultrasound.[7,8] Erdogan et al. found considerable contributions of LIPU (1.5 MHz) to bone healing in mandibular fractures.[9]

These previously reported studies used 1.5 MHz ultrasound in the maxillofacial area.[1,6–9] Although some compared the bone healing process based on weeks post application,[1,6,8] the effect of different frequencies has not been evaluated in this region. Taking into account the reported positive influences of ultrasound on the bone healing processes with the disturbed function of impaired bone healing in large maxillofacial defects, it was decided to investigate the potential of LIPU to stimulate mandibular bone defect healing at two different frequencies, over 2 and 4 weeks, in a rabbit model.

Materials and methods

The study was approved by the university ethics committee. Fifty-six mature male Dutch rabbits weighing a mean 2.4 ± 0.2 kg and aged a mean 18 ± 2 months were included (Table 2). Unilateral mandibular defects were created in all animals and they were subsequently divided into three treatment groups, which were subdivided further into six subgroups. The first two subgroups of animals (G1 and G2, $n = 11$ in each) received 1 MHz ultrasound treatment daily, 10 min/day, for 2 weeks (G1) or 4 weeks (G2). The second two subgroups (G3 and G4, $n = 11$ in each) received 3 MHz ultrasound with the same protocol for 2 weeks (G3) or 4 weeks (G4). Animals in the two control groups (G5 and G6, $n = 6$ in each) received sham application for 2 weeks (G5) or 4 weeks (G6).

Table 2. Study groups for the application of low-intensity pulsed ultrasound (LIPU) on mandibular defects.

Group	LIPU	Time of sampling after LIPU, weeks	Number of rabbits
G1	1 MHz	2	11
G2	1 MHz	4	11
G3	3 MHz	2	11
G4	3 MHz	4	11
G5 (control 1)	None	2	6
G6 (control 2)	None	4	6

Surgical procedure

All rabbits underwent general anaesthesia by intramuscular injection of 44 mg/kg ketamine 10% (Alfasan International BV, Woerden, the Netherlands) and 8 mg/kg xylazine 2% (Alfasan International BV). After placing the animal in a supine position, the mandible was exposed with a 3-cm incision in the diastema area. A unilateral extraoral submandibular approach was used and a 5 mm × 2 mm × 1 mm bone defect was created 5 mm medial to the mental foramen on one side using a round bur (ELA Carbide; Emil Lange – Zahnbohrerfabrik e.K., Engelskirchen, Germany); all layers were then sutured (Fig. 1).

Postoperative care

An analgesic (flunixin 5%, 0.15 mg/kg; Erfan Daru Pharmaceutical, Tehran, Iran) and an antibiotic (penicillin 22,000 IU/kg; Erfan Daru Pharmaceutical) were administered intramuscularly preoperatively and twice per day for four postoperative days. The rabbits were housed in separate cages and fed soft food for 1 week. After the first week, a normal diet was resumed. Food and water intake and the weights of the animals were monitored and recorded daily. Animals that had a weight loss of more than 20% of their initial body weight were excluded from the study.

Ultrasound application

On the second postoperative day, the application of LIPU was started for the animals in the four experimental groups. A commercially available therapeutic ultrasound device (new version 215× class 1 type BF; Nvin Medical Engineering Co., Isfahan, Iran) was used for the ultrasound