LOW-INTENSITY TRANSOSSEOUS ULTRASOUND ACCELERATES OSTEOTOMY HEALING IN A SHEEP FRACTURE MODEL

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Background: Low-intensity transcutaneous ultrasound can accelerate and augment the fracture-healing process. The aim of this study was to investigate the effect of transosseous application of low-intensity ultrasound on fracture-healing in an animal model.

Methods: A midshaft osteotomy of the left tibia was performed in forty sheep. An external fixator was used to stabilize the osteotomy site. A thin stainless-steel pin was inserted into the bone, 1.0 cm proximal to the osteotomy site. Ultrasound was transmitted through the free end of this pin, with a PZT-4D transducer. In twenty animals, the treated limb received a 200-μsec burst of 1-MHz sine waves repeated at 1 kHz with an average intensity of 30 mW/cm^2 for twenty minutes daily. Twenty other animals underwent the same surgery but did not receive the ultrasound (controls). Animals were killed at seventy-five and 120 days postoperatively. Radiographic evaluation was performed every fifteen days. Mechanical testing and quantitative computed tomography were performed after death.

Results: Fractures treated with ultrasound healed significantly more rapidly, as assessed radiographically, than did the controls (seventy-nine compared with 103 days, p = 0.027). On day 75, the mean cortical bone mineral density (and standard deviation) was 781 ± 52 mg/mL in the treated limbs compared with 543 ± 44 mg/mL in the control group (p = 0.014), and the average ultimate strength (as assessed with a lateral bending test) was 1928 ± 167 N in the treated limbs compared with 1493 ± 112 N in the control group (p = 0.012). No significant differences were noted on day 120.

Conclusions: This study demonstrated that low-intensity transosseous ultrasound can significantly accelerate the fracture-healing process, increase the cortical bone mineral density, and improve lateral bending strength of the healing fracture in a sheep osteotomy model.

Clinical Relevance: Transosseous application of low-intensity ultrasound at close proximity to the fracture site may enhance the mechanical properties of the fracture callus and reduce the time to fracture-healing. However, further investigation is needed to establish the safety and efficacy of the technique.

Ultrasound is currently applied for diagnostic and therapeutic purposes[1]. Ultrasonic intensities for therapeutic and surgical procedures are high (1 to 50 W/cm^2) and can cause considerable tissue-heating[2]. In contrast, the intensity for diagnostic procedures is low (1 to 50 mW/cm^2) and is considered to be nonthermal and safe[3,4].

To our knowledge, low-intensity ultrasound was first used to enhance fracture-healing by Duarte[5], who reported good results. The positive effect of low-intensity ultrasound on the rate of osseous repair has been well documented in several animal[6-9] and clinical[10-12] studies.

Animal studies have demonstrated that ultrasound treatment substantially increases the strength of a healing fracture. Pilla et al.[7] reported that the mechanical properties of a healing rabbit fibular fracture were accelerated by a factor of nearly 1.7 after application of low-intensity ultrasound. Wang et al.[8] as well as Yang et al.[9] used a rat bilateral femoral fracture model with application of ultrasound to one of the two fractures. Both studies showed that the average maximum torque and torsional stiffness were significantly greater in the ultrasound-treated limbs than in the control limbs.

The ultrasound intensity level in most reported series has

TABLE I Cortical Bone Mineral Density, Measured with Quantitative Computed Tomography, of the Osteotomized and Control Tibiae in Both Groups

	Cortical Bone Mineral Density* *(mg/mL)*				
	Osteotomized Tibiae		Intact Tibiae		
Postoperative Day	Treatment Group	Control Group	Treatment Group	Control Group	P Value†
75	781 ± 52	543 ± 44	1375 ± 45	1380 ± 38	a: 0.014 b: 0.001 c: 0.001
120	941 ± 81	906 ± 69	1363 ± 30	1372 ± 32	a: 0.78 b: 0.001 c: 0.001

*The values are given as the mean and standard deviation. †As determined with analysis of variance. a: treatment group compared with control group; b: intact tibia compared with osteotomized tibia in treatment group; and c: intact tibia compared with osteotomized tibia in control group.

ranged from 30 to 100 mW/cm^2—in the range of intensities used for diagnostic imaging—in order to avoid harmful effects. In most series, the fracture sites were exposed to ultrasound for twenty minutes per day. In the above-mentioned studies, the mechanical energy of the ultrasound was transferred to the tissues through a transducer, which was applied to the skin over the site of the fracture. The same technique was used in clinical studies[11,12]. However, ultrasonic energy has to pass through skin, fat, muscle, and hematoma before reaching the fracture site, and there is absorption of the ultrasonic energy as the ultrasound wave is transmitted through these tissues.

Our hypothesis was that application of the ultrasound directly to bone, bypassing the overlying tissues, would be a feasible, safe, and effective way to enhance bone-healing. The goals of our study were (1) to develop a novel, transosseous method for ultrasound transmission, and (2) to test the safety and efficacy of this method in accelerating fracture-healing.

Materials and Methods

Experimental Design and Animal Model

Forty skeletally mature sheep weighing 45 to 55 kg were randomly assigned to two groups of equal size: the ultrasound group and the control group. An osteotomy was created in the tibia of the left hindlimb and was stabilized with an external fixator. Transosseous ultrasound was applied to the osteotomy site in the twenty animals in the ultrasound group, whereas no other intervention was performed in the twenty animals in the control group.

Ten sheep from each group were killed on postoperative day 75, and ten were killed on postoperative day 120. Both the operatively treated left tibia and the contralateral tibia were harvested, and quantitative computed tomography and subsequent biomechanical testing were performed.

The research protocol was reviewed and approved by the Animal Committee of the University of Ioannina. All animals appeared to be healthy and were housed individually with appropriate husbandry conditions before and after the procedure.

Transosseous Ultrasound Transmission Model

Transosseous ultrasound transmission was performed with a method that was developed for the current investigation. A custom-made stainless-steel pin, measuring 40 mm in length and 2 mm in diameter, with one sharp and one blunt end, was used. The blunt end was specially designed to accept and firmly hold a PZT-4D transducer (Morgan Matroc, Bedford, Ohio) in place to avoid dislodgment during movement of the animals. This pin-transducer apparatus enables the ultrasound energy to be transmitted through the free end of the pin to the sharp end, which is inserted 1.0 cm proximal to the osteotomy site.

The pin-transducer apparatus was tested at the Ultrasound Laboratory of the Department of Physics of the Uni-

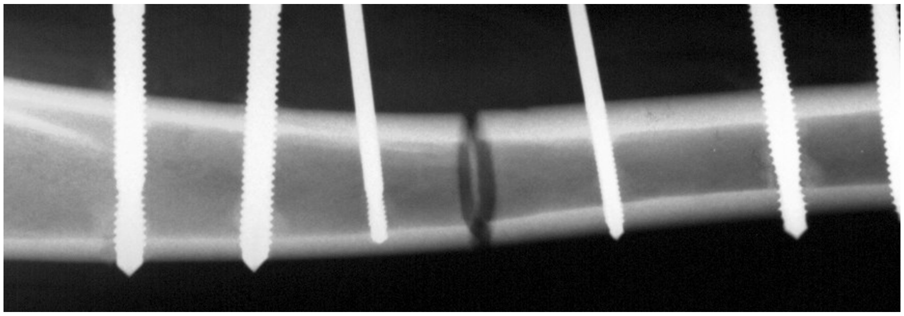


Fig. 1

Postoperative radiograph. The second smooth pin (also 1 cm from the osteotomy site) was used only to evaluate the fracture-healing with ultrasound.

TABLE II Ultimate Strength (Fracture Load) of the Osteotomized and Control Tibiae in Both Groups

	Ultimate Strength* (N)				
	Osteotomized Tibiae		Intact Tibiae		
Postoperative Day	Treatment Group	Control Group	Treatment Group	Control Group	P Value†
75	1928 ± 167	1493 ± 112	2412 ± 57	2410 ± 67	a: 0.012 b: 0.001 c: 0.001
120	2361 ± 154	2286 ± 135	2398 ± 81	2405 ± 93	a: 0.29 b: 0.49 c: 0.635

*The values are given as the mean and standard deviation. †As determined with analysis of variance. a: treatment group compared with control group; b: intact tibia compared with osteotomized tibia in treatment group; and c: intact tibia compared with osteotomized tibia in control group.

versity of Ioannina to ensure that no substantial loss of ultrasound energy occurred at the connection site or during propagation of the ultrasound wave through the pin. The 1-cm distance of the pin from the osteotomy site was selected to maximize the energy absorbed at the osteotomy site.

Operative Procedure and Ultrasound Application

Anesthesia was achieved with use of ketamine (10 mg/kg) and xylazine (3 mg/kg). The hindlimbs were shaved, scrubbed with Betadine (povidone-iodine, 10% solution), and draped. Under aseptic conditions, a Monotube unilateral external fixator (Howmedica Jaquet, Geneva, Switzerland) was mounted on the anterolateral aspect of the left tibia with use of a four-pin technique. The tibia was exposed subperiosteally, and a mid-diaphyseal osteotomy was performed, starting with multiple adjacent 2.0-mm drill holes and completing the osteotomy with a bone chisel.

The custom-made pin was applied 1 cm proximal to the osteotomy site and perpendicular to the anterolateral aspect of the cortex in all animals. The pin was inserted into the medullary canal, and it engaged the opposite cortex for additional stability. A second pin was also inserted, 1 cm distal to the osteotomy site, but this was used only to monitor fracture-healing with ultrasound in both groups. In the treatment group, ultrasound energy was transmitted through the free end of the proximal pin with a PZT-4D transducer. Radiographs were used to make certain that the osteotomy was transverse and not displaced (Fig. 1). The skin and fascia were closed with absorbable sutures.

The sheep were allowed unrestricted walking in their cages after they recovered from the anesthesia, and they were observed daily for activity, wound-healing, and development of pin-track infection.

The animals in the treatment group received ultrasound exposure for twenty minutes daily, starting on the first postoperative day, until they were killed. The ultrasound signal consisted of a 200-μsec burst sine wave of 1.0 MHz repeating at 1.0 kHz. The intensity was 30 mW/cm^2 spatial average and temporal average.

Radiographic Assessment of Healing

Anteroposterior and lateral radiographs were made every fifteen days for all animals. However, only the radiographs of animals that were killed on postoperative day 120 were used for the radiographic assessment of healing, in order to allow adequate time for healing in both groups.

We used the method of Heckman et al.[11] for radiographic assessment of healing. At each time-point, four cortices (two on the anteroposterior radiograph and two on the lateral radiograph) were evaluated for cortical bridging. All radiographs were assessed independently by a radiologist (A.H.K.) and an orthopaedic surgeon (C.G.Z.), who were blinded to the treatment group. Radiographic healing was defined as the time at which three of the four cortices were bridged.

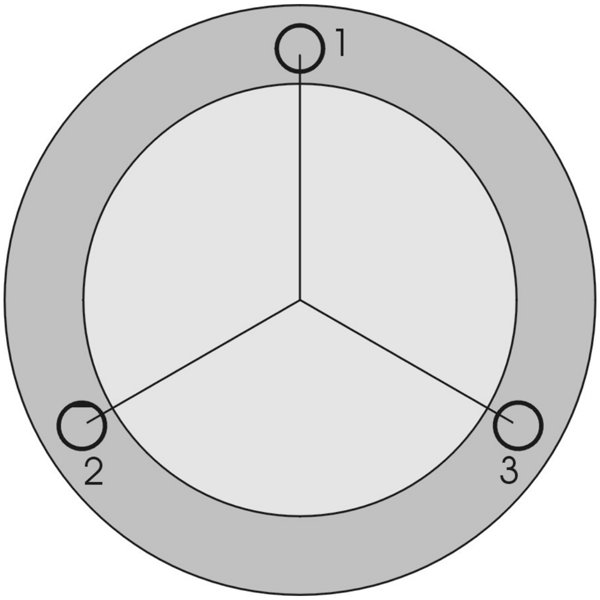


Fig. 2

The bone density in regions 1, 2, and 3, which are located in the cortical zone, was measured, and then the average was calculated.

TABLE III Stiffness of the Osteotomized and Control Tibiae in Both Groups

	Stiffness* (N/mm)				
	Osteotomized Tibiae		Intact Tibiae		
Postoperative Day	Treatment Group	Control Group	Treatment Group	Control Group	P Value†
75	98 ± 35	70 ± 19	138 ± 22	141 ± 17	a: 0.034 b: 0.018 c: 0.001
120	128 ± 32	119 ± 28	145 ± 8	139 ± 25	a: 0.721 b: 0.580 c: 0.612

*The values are given as the mean and standard deviation. †As determined with analysis of variance. a: treatment group compared with control group; b: intact tibia compared with osteotomized tibia in treatment group; and c: intact tibia compared with osteotomized tibia in control group.

Specimen Harvest

At the predetermined time-points, the sheep were killed, after induction of general anesthesia with sodium pentobarbital, with intravenous administration of potassium chloride. Soft tissues were dissected from both tibiae of each animal, and the denuded tibiae were stored in 70% ethanol for the purposes of quantitative computed tomography scanning and mechanical testing.

Quantitative Computed Tomography and Biomechanical Testing

Quantitative computed tomography (Philips LX Plus; Philips Medical Systems, Best, The Netherlands) was performed at the level of the osteotomy. At each cross section, the density of three regions in the cortical zone was measured (Fig. 2), and the average of the three cortical values was described as cortical bone mineral density. For mechanical testing, all specimens were tested in a computerized testing machine (Karl-Frank, Bern, Switzerland) applying a three-point bending test at room temperature[13]. All bones were loaded with a low strain rate (0.05 mm/sec) until failure, and ultimate strength (N) and stiffness (N/mm) were determined from the load-displacement curve. The bending load was applied to the specimen at the site of the osteotomy and in the sagittal plane. All specimens were fixed on the three-point bending apparatus, and the span of loaded bone was 8 cm to guarantee that 90% of the flexion of the bone was due to bending and not to torsion[13].

Statistical Analysis

The Mann-Whitney two-tailed nonparametric test was used to compare the median times to healing between the two groups. Analysis of variance was used to assess densitometric and biomechanical data, followed by the Tukey test for pairwise comparison between groups. Significance was accepted at $p < 0.05$.

Results

All animals survived until the end of the study. A minor external-fixation pin-track infection developed in five animals (three from the ultrasound group and two from the control group), and all were easily treated with local care of the pin track. No pin had to be removed, and osteomyelitis did not develop in any of the animals. No infection was noted at the sites of the pins used for ultrasound transmission.

There was a significant difference between the ultrasound and control groups with regard to cortical bone mineral density at seventy-five days postoperatively. The mean cortical bone mineral density (and standard deviation) was 781 ± 52 mg/mL in the treatment group and 543 ± 44 mg/mL in the control group ($p = 0.014$, analysis of variance) (Table I). At seventy-five days, central callus resorption and remodeling of the periosteal callus had started in the treatment group. In contrast, the callus in the control group covered the entire cross section, including the marrow canal, without remodeling of the cortex area (Figs. 3-A and 3-B). However, at 120 days, the difference between the treatment and control groups was not significant (941 ± 81 mg/mL and 906 ± 69 mg/mL, respectively; $p = 0.78$, analysis of variance) (Table I).

Mechanical testing with the lateral bending test on postoperative day 75 revealed significantly greater ultimate strength and stiffness in the treated limbs compared with the control limbs. The average ultimate strength was 1928 ± 167 N in the treatment group and 1493 ± 112 N in the control group ($p = 0.012$, analysis of variance) (Table II). Nevertheless, the ultrasound-treated limbs as well as the control limbs were significantly weaker than the intact tibiae ($p = 0.001$) at that time. As was the case with the cortical bone mineral density, by postoperative day 120 the difference in ultimate strength between the treatment and control groups was not significant (2361 ± 154 N and 2286 ± 135 N, respectively; $p = 0.29$, analysis of variance). Similarly, the stiffness in the treated limbs was significantly greater than that in the control limbs on postoperative day 75 ($p = 0.034$), but there was no significant difference between the groups on postoperative day 120 (Table III).

The radiographs of the ten animals from the treatment group and the ten from the control group that were killed on postoperative day 120 were analyzed to assess healing time. Although all fractures healed by 120 days in both groups, there was a significant difference between the two groups with respect to the time of healing. The median time for radiographic healing (bridging of three of the four cortices), as assessed by the orthopaedic surgeon, was seventy-nine days (range, sixty to ninety days) for the treatment group and 103 days (range, seventy-five to 105 days) for the control group (p = 0.027, Mann-Whitney test). As assessed by the radiologist, on review of the same radiographs, the corresponding times were eighty-eight days (range, sixty to 105 days) compared with 109 days (range, seventy-five to 120 days) (p = 0.038, Mann-Whitney test) (Figs. 4-A through 5-B).

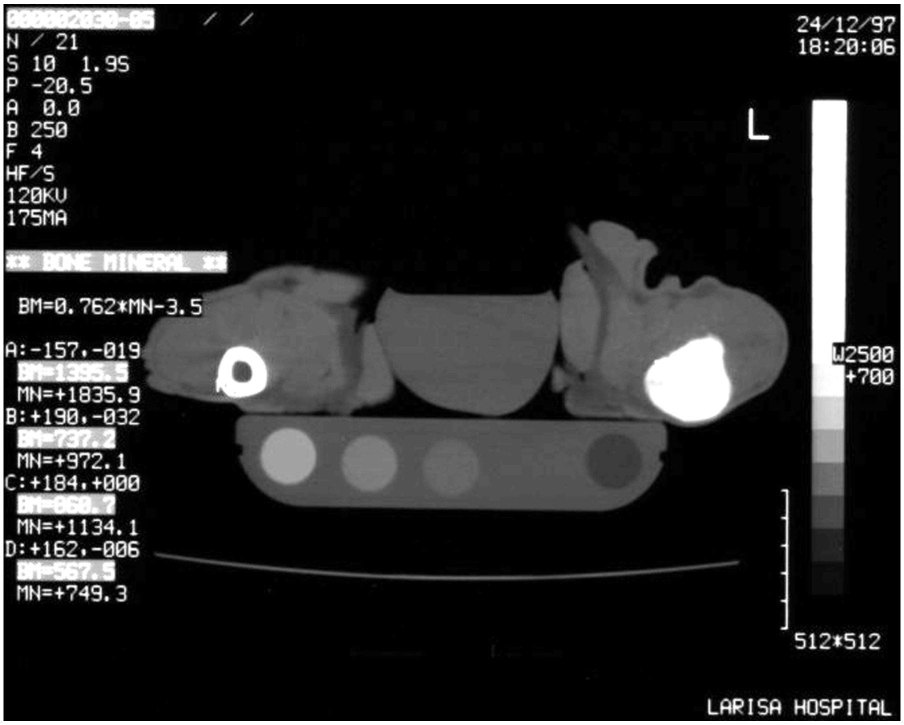
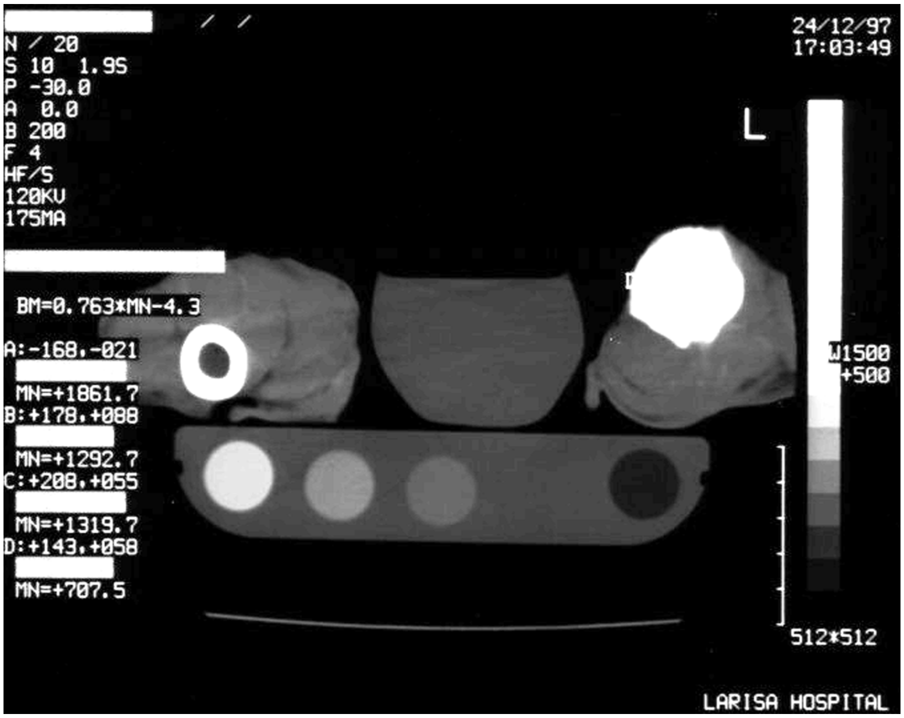


Fig. 3-A

Figs. 3-A and 3-B Computed tomography scans at the osteotomy site seventy-five days postoperatively. **Fig. 3-A** The control group. Abundant callus formation covering the entire osteotomy area without evidence of remodeling is seen.

Fig. 3-B

The treatment group. Formation of cortical bone and remodeling of the callus with reconstitution of the marrow canal are seen.



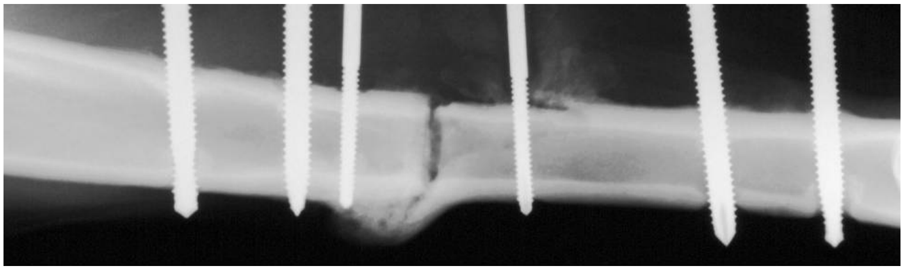


Fig. 4-A

Figs. 4-A and 4-B Radiographs of osteotomized tibiae sixty days postoperatively. **Fig. 4-A** The control group. Callus formation around the osteotomy site is poor and obvious only in a small area.

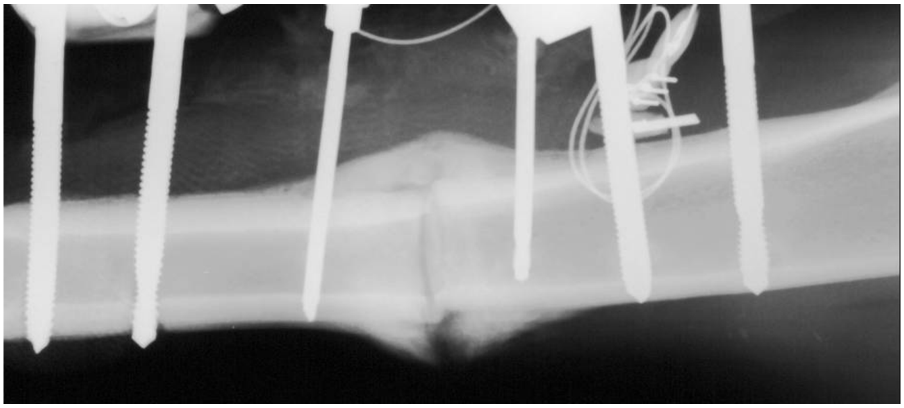


Fig. 4-B

In contrast, in the treatment group the callus is more dense and homogeneous with a large diameter.

Discussion

Many basic science[5-9] and clinical studies[10-12] have shown that low-intensity ultrasound can accelerate and augment the fracture-healing process. The mechanisms by which low-intensity ultrasound interacts with tissue and accelerates fracture-healing remain unknown. It has been hypothesized that when ultrasound passes through tissues, absorption of ultrasonic energy occurs at a rate proportional to tissue density[14]. This energy absorption has been reported to increase cell metabolic activity[3], stimulate vascular activity[14,15], increase gene expression[9,16], enhance calcium incorporation at the fracture site[17], offer a beneficial mechanical stimulus in the healing callus[18], and enhance fracture-healing.

At interfaces of tissues with different densities, such as at bone-callus surfaces, much of the incident radiation energy is reflected, resulting in complex gradients of acoustic pressure through the tissue and reduction of the transmitted energy[19]. Because of this, we were motivated to investigate the feasibility of direct transmission of ultrasound through bone to the callus area and to determine whether the transosseous method could be an alternative to the conventional transcutaneous technique to promote fracture-healing. To our knowledge, we are the first to investigate the effect of transosseous low-intensity ultrasound on fracture-healing.

Our custom-made apparatus was able to transmit ultrasound waves directly to the callus area, which enhanced the healing process. Insertion of the apparatus pin in close proximity to the osteotomy site did not have any adverse effects. We were concerned that the pin could be a source of infection. Although five minor external-fixation pin-track infections developed in the animals, they did not compromise fracture-healing.

Transosseous application of low-intensity ultrasound enhanced bone-healing effectively in this animal model, as demonstrated by the biomechanical, quantitative computed tomography, and radiographic data. The biomechanical testing demonstrated that low-intensity transosseous ultrasound is capable of significantly accelerating the biomechanical properties of a healing osteotomy site in a controlled fracture model. On postoperative day 75, the average stiffness of the treated fractures was 40% greater than that of the control fractures. Previous investigators[9,20] have attributed this increase to stimulation of chondrogenesis and cartilage hypertrophy by ultrasound exposure, resulting in an earlier onset of endochondral bone formation and subsequently in increased fracture callus strength in the early phases of healing. However, by postoperative day 120, there was no significant difference between our two groups.

Analysis of the callus region with quantitative computed tomography also revealed a significant difference between the ultrasound and control groups with regard to cortical bone mineral density on postoperative day 75. Computed tomography allows three-dimensional imaging and an objective estimation of the callus region. Our analysis of the computed tomography images showed that the stage of callus healing differed between the two groups. Reconstitution of the marrow canal and cortical remodeling had started in the limbs treated with the low-intensity ultrasound, whereas cancellous bone was more abundant and the cortical remodeling was not obvious in the control limbs. Thus, acceleration of the healing process was observed in the treated limbs. This finding is in agreement with that of Azuma et al.[20], who found, with densitometric methods, that low-intensity ultrasound increased bone mineral density and accelerated the overall

Fig. 5-A

Fig. 5-B

Figs. 5-A and 5-B Radiographs of osteotomized tibiae 120 days postoperatively, after removal of the fixator and ultrasound pins. The tibia in both the control group (Fig. 5-A) and the treatment group (Fig. 5-B) has dense, thick cortices in the osteotomy area, with the same diameter as that of the original tibia and almost complete bone-remodeling.

endochondral ossification process. However, by postoperative day 120, we observed no significant differences between our control and treatment groups.

To assess the rate of acceleration of fracture-healing, we used radiographic criteria similar to those employed by Heckman et al.[11] and Kristiansen et al.[12]. We found that transosseous low-intensity ultrasound significantly reduced the median time to radiographic healing (from 103 days in the control group to seventy-nine days in the ultrasound treatment group, $p = 0.027$) as assessed by the orthopaedic surgeon, but all fractures in both groups had healed by postoperative day 120.

Two double-blinded, randomized, placebo-controlled clinical studies[11,12] showed that transcutaneous low-intensity ultrasound significantly reduced the time for radiographic healing. Heckman et al.[11] found that ultrasound led to a 40% decrease in the time for radiographic healing of tibial fractures treated with a cast, with the ultrasound-treated fractures healing in eighty-nine days compared with 148 days in the control group ($p < 0.001$). Similarly, in another study of the time to union of distal radial fractures treated with a cast, Kristiansen et al.[12] found that ultrasound shortened the time to healing by 38%.

The results of our study should be viewed with caution. We utilized a sheep osteotomy model, which differs from the approach in the two above-mentioned studies. The sheep model has limited applicability to human fractures, so caution should be used in extrapolating our results to humans. Moreover, the fact that pin-track infections were only a minor problem in sheep does not guarantee safety in clinical practice because, in humans, the pin may need to be in place for a longer period of time and infectious complications could develop. As a consequence, the clinical benefits of the transosseous technique remain to be established. The current investigation should be viewed as a preliminary experimental study, and the results should not be applied to human clinical practice before additional investigations address the aforementioned reservations. However, previous studies have shown that transcutaneous ultrasound was effective in both animals[5-9] and humans[10-12].

Another limitation of our study is that we did not compare transosseous and transcutaneous methods. However, we wanted to first establish the feasibility, safety, and efficacy of the transosseous method before proceeding with a direct comparison. Our study is also limited by the lack of a histological analysis, which could have provided information regarding canal reconstitution and cortical remodeling. Quantitative computed tomography extraction of the relevant morphometric parameters regarding the cancellous and cortical bone compartments would have also been beneficial by delineating bone structure at the healing osteotomy site.

Our study suggests that transosseous application of low-intensity ultrasound close to the fracture site enhances the mechanical properties of the fracture callus, increases the cortical bone mineral density of the regenerated bone, and reduces the time to healing. Additional studies are needed to determine whether this invasive method is advantageous in comparison with the conventional transcutaneous method and to clarify the technical details of optimal application. ■

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