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Original Article

Stimulation of Fracture Healing in a Canine Ulna Full-defect Model by Low-intensity Pulsed Ultrasound

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Because no report has been issued on the healing effects of low-intensity pulsed ultrasound on moderate to large fracture gaps, we performed an experimental study using a canine ulna full-defect model. Ten mongrel male dogs were divided into two groups: a small defect group and large defect group. The defects were made on the middle one third of both ulnae and one side only was randomly selected for ultrasound sonication, at 1 MHz, 200 microsecond bursting sine wave in 50 mW/cm^2 spacial average and temporal average. Sonication was started on the day after surgery and applied for 15 minutes once a day for six days a week. In the small defect group, the means of the radiologic scores, as described by Lane and Sandhu, were 0.6, 4.4, and 8.4 in the control side and 1.8, 6.0, and 10.4 in the treatment side one, three, and five months after the operation, respectively (p=0.0372). In the large defect model, the corresponding means were 2.2, 3.4, and 6.0 in the control side and 3.3, 5.4, and 9.2 in the treatment side (p= 0.009). Low-intensity pulsed ultrasound enhanced new bone formation in small and large full-defects and decreased the incidence of nonunion in the large defect model.

Key Words: Fracture, healing, ultrasound, stimulation, ulna, canine

INTRODUCTION

Low-intensity pulsed ultrasound has been proven to be an effective way of accelerating fracture healing in fracture models and clinical trials.[1-7] It is an only physical method, and has been approved by the Food and Drug Administration (FDA) for the promotion of fracture healing in fresh fractures (October 1994) and nonunions (February 2000).[8] In our previous study, we used a simple osteotomy model of the canine distal ulna to evaluate the effectiveness of low-intensity pulsed ultrasound on fracture healing.[5,9] Radiological healing scores and mean isotope uptakes were significantly higher in the treated side. Based on the cumulative union rate curve, fracture healing was stimulated and union was obtained one month faster in the treated side than in the control side. Under clinical condition, however, we encountered moderate to large fracture gaps more frequently than simple fracture lines as an osteotomy model. The mode of treatment has also altered from anatomical reduction and rigid internal fixation to biological fixation, which emphasizes the bypassing of comminuted fragments, leaving the fracture gap undisturbed.[10] Since there has been no experimental report concerning the effectiveness of low-intensity pulsed ultrasound on moderate to large fracture gaps, which could lead to a delay in the normal healing process or nonunion, we performed an experimental study using a canine ulna full-defect model.

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MATERIALS AND METHODS

Canine ulna full-defect model

Ten mongrel male dogs (body weight 7 - 8 kg) were divided into two groups. A radiograph of the knee was taken to confirm closure of the epiphysis. Under general endotracheal enflurane anesthesia, both forelegs were prepared and draped in a sterile manner. A medial longitudinal incision about 10 cm was made on the middle one third of both forelegs. The soft tissue and periosteum of the ulna was incised and retracted, and a double osteotomy was performed at the middle of both ulnae using a Gigli saw. The size of the full-defect (bone resection) was determined by the width of the diaphysis in the operation field. In group 1 (the small defect group), the defect was one half (½) of the width of the diaphysis. In group 2 (the large defect group), the defect was one and half (1½) times the width of the diaphysis. Each group consisted of five dogs. To perform osteotomy at the ulna, bone clamps were firmly attached to both end of the osteotomy site. A Gigli saw was then passed under the ulna and pulled along the edge of the bone clamp. Periosteum was intermittently sutured with 3-0 Vicryl suture (Johnson and Johnson, Edinburgh, U.K.), and the skin was closed with a subcuticular running 3 - 0 Vicryl suture. Cefalozin (20 mg/kg) was injected to the buttock twice a day for 3 days. The dogs were kept in cages without any restriction in motion and fostered with Jerony pet food (Cheil Jedang, Incheon, Korea) at a constant temperature and humidity in an animal laboratory. We randomized the study (treatment) side of the foreleg in each animal. Sonication was started on the day following surgery and applied for 15 minutes once a day for six days a week during the experiment. Sonication gel was applied on the skin over the defect to avoid air collection between skin and the transducer. The transducer was fixed to the foreleg by a specially made band. The same procedure was performed on the control side but power was not applied.

Characteristics of the ultrasound wave

The ultrasound fracture stimulator was made and provided by the Medison company (Seoul, Korea). We used a transducer with a zirconium head, two centimeters in diameter. The ultrasound was of 1 MHz, 200 microsecond bursting sine wave in 50 mW/cm^2 spacial average and temporal average.

Evaluation of the union

Serial antero-posterior and lateral radiographs of both ulna were taken one, three, and five months after the operation. If the defect was not completely bridged with regenerating bone, it was defined as a nonunion. We evaluated the healing process using the radiological criteria described by Lane and Sandhu for defect models (Table 1).[11] The radiologic scores were analyzed by Repeated Measures Analysis of Variance. The difference between the control and the study sides was regarded as significant when the *p* value was less than 0.05.

Table 1. Radiologic Scoring System by Lane and Sandhu

Bone formation	
No evidence of bone formation	0
Bone formation occupying 25% of defect	1
Bone formation occupying 50% of defect	2
Bone formation occupying 75% of defect	3
Full gap bone formation	4
Union	
Full fracture line	0
Partial fracture line	2
Absent fracture line	4
Remodeling	
No evidence of remodeling	0
Remodeling of intramedullary canal	2
Full remodeling of cortex	4

RESULTS

All dogs survived for 5 months and were evaluated, and no infection occurred.

Group 1 : small defect model

The mean radiologic score were 0.6, 4.4, and 8.4 in control side and 1.8, 6.0, and 10.4 in treatment side one, three, and five months after operation, respectively (Fig. 1). Moreover, the difference between the control and the treatment side was statistically significant (p=0.0372). There was no case of nonunion in either the treatment or the control side. Remodeling of the cortex was found in four cases on the treatment side and in one case on the control side. The effect of ultrasound appeared during the early stage of healing and persisted until the end of the study (Fig. 2).

Group 2 : large defect model

The mean radiologic score were 2.2, 3.4, and 6.0 in the control side and 3.3, 5.4, and 9.2 in the treatment side one, three, and five months after the operation, respectively (Fig. 3). The difference between the control and the treatment side was statistically significant (p=0.009). Three control side cases of five large defects showed nonunion (Fig. 4). All defects in the study side united but remodeling was delayed. Healing of in the large defect group was delayed compared to that of the small defect group ($p < 0.001$).

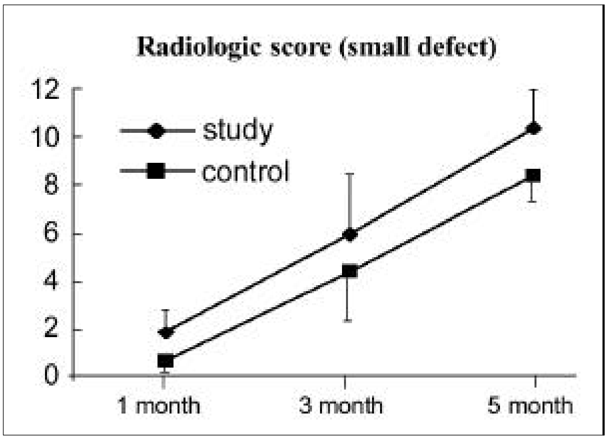

Fig. 1. Radiologic score of the small defect group in a canine ulna full-defect model. The means were higher in the treatment side than in the control side (p=0.0372, repeated measures ANOVA). The effect of ultrasound appeared during the early stages of healing and persisted until the end of the study. The vertical bar indicates one standard deviation.

DISCUSSION

Fracture healing is a specialized type of wound-healing response in which the regeneration of bone leads to a restoration of skeletal integrity. A complex biological healing process that involves many cell type and genes.[8,12-14] Approximately 5 to 10% of the fractures, don't consolidate in expected time. The main causes of delayed or impaired fracture healing are inadequate immobilization, large gap, smoking, and soft tissue injury, which results in a decrease in vascular invasion. Scantiness of soft tissue and an open wound on the medial aspect of the tibia frequently result in delayed healing compared to the lateral cortical gap.[15] Many mechanical and physical methods have been introduced to enhance fracture healing for those fractures that are associated with the above mentioned risk factors. Early micromotion, weight bearing, bioelectro-magnetic field, and ultrasound have all been proven to enhance fracture healing. An external fixator is necessary for the application of early micromotion and good stability is a prerequisite of early weight bearing.[16] Ultrasound sonication is the only noninvasive method, and furthermore, it does not endanger fracture configuration during the early fracture healing stage.

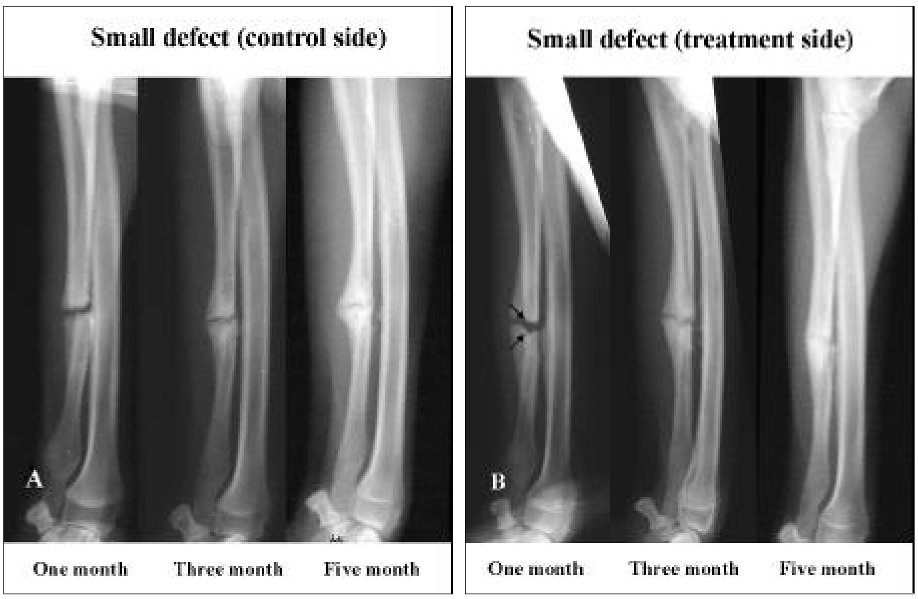

Fig. 2. Radiographs of the small defect group in a canine ulna full-defect model. (A) control side, (B) treatment side. The defect was filled up with new bone at three months and remodeling was found at five months after operation in the study side. The arrows indicate osteotomy sites.

Ultrasound has been applied in rehabilitation clinics for a long time. Acoustic energy, of 1 to 3 W/cm^2, is transmitted to the deep tissue and generates heat, thus decreasing joint stiffness and muscle spasm. Corradi and Cozzolono[1] were the first investigators to demonstrate that low-energy ultrasound could stimulate the formation of bone callus.[1] Many experimental studies have confirmed that ultrasound of 30 - 50 mW/cm^2 spacial average and time average, 0.5 - 1.5 MHz, and bursting pulse pattern with a pulse width of 200 microsecond in 1 KHz repetition rate is regarded as the most effective wave pattern in terms of accelerating fracture healing. Based upon two major double blinded clinical studies, the FDA approved the use of ultrasound for the enhancement of fresh fracture healing. Ultrasound was found to diminish the deteriorative effect of smoking and to decrease the incidence of nonunion. Its application has been extended to treatment of nonunion,[17,18] and experimental studies have shown that ultrasound enhances bone maturation in distraction osteogenesis.[19]

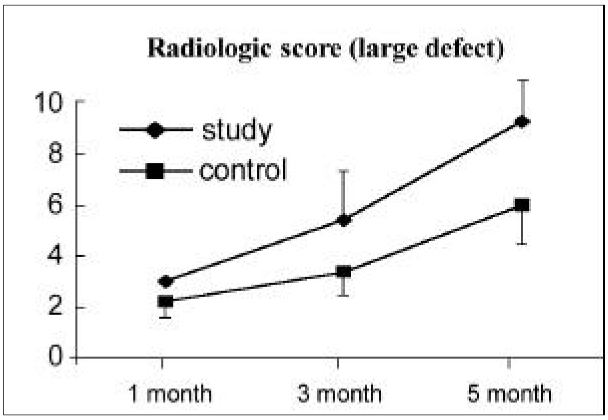

Fig. 3. Radiologic score of the large defect group in a canine ulna full-defect model. The means were higher in the treatment side than in the control side (p=0.009, repeated measures ANOVA). Three control side cases of five large defects showed nonunion. Healing of the large defect group was delayed compared to the small defect group (p < 0.001). The vertical bar indicates one standard deviation.

Our previous study showed accelerated fracture healing in a canine ulna osteotomy model by low-energy pulsed ultrasound.[5] To the best of our knowledge this was the first report performed in a canine model. Since the physiology and metabolism of canine bone are much closer to human bone than the rabbit or the rat, results using a canine model facilitate clinical trials. In double blinded clinical studies, ultrasound was found to shorten the union time of tibial and Colles' fractures by about 24 - 37 days on average.[6,7] In our previous osteotomy model, the union time was shortened by about 1 month by sonication. Our current study was designed to mimic the clinical situation in which reduction and internal fixation of comminuted fracture fragments is difficult to perform unless extensive stripping of

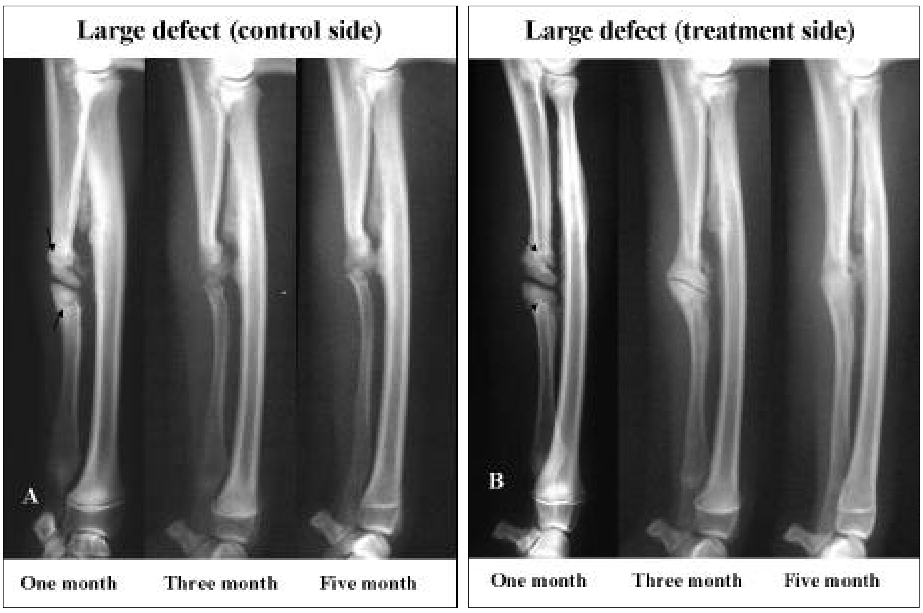

Fig. 4. Radiographs of the large defect group in a canine ulna full-defect model. (A) control side, (B) treatment side. The defect was nearly filled with new bone at three months and remodeling was underway five months after operation in treated animals. Fracture healing stopped at three months and resulted in nonunion in the control side. The arrows indicate osteotomy sites.

the soft tissue is performed. Biological fixation rather than anatomical reduction and rigid internal fixation is preferred for these fractures. The technique respects vascular supply to the fragments and the surrounding soft tissues including the periosteum. In the present study, we didn't resect the periosteum over the defect, because it is believed to be the major responsive element in ultrasound treatment and in the normal fracture healing process. We believe that a low rate of nonunion even in the control side may be attributed to the preservation of the periosteum.

The mechanism of fracture healing acceleration by low-energy pulsed ultrasound is not clear. Many investigations have shown that ultrasound enhances angiogenensis,[20] calcium incorporation in differentiating cells,[21] and aggrecan gene expression in the early fracture callus.[13,14] Based upon the available evidence, the main targets of ultrasound seems to be the differentiating cells in the soft callus and periosteum, which results in a proliferation and hypertrophy of the chondrocytes and a lager callus formation.[13,14] Since area moment of inertia increases to the fourth of the radius, the effect of an increase in the size of the external callus is remarkable. Therefore, the application of ultrasound is preferable when fracture healing is mediated by endochondral ossfication. In the defect model, the defect is filled by organized hematoma and the subsequent invasion of vessels and chondrogenesis. Our data confirm early bone formation in the defect and maturation in ultrasonically treated cases. In the canine model, the radiologic score at one month indicated early bone formation, at three months union, and at five months remodeling. In small defect group, the defects were healed and remodeling had started at five months. And, in the large defect group, the defects were united but remodeling was not evident in cases at five months in the treated animals. Moreover, ultrasound decreased the incidence of nonunion.

In conclusion, low-intensity pulsed ultrasound enhanced new bone formation in small and large full-defects of the middle of the canine ulna diaphysis, and decreased the incidence of nonunion in a large defect model.

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