Ultrasound in Med. & Biol., Vol. 30, No. 3, pp. 389–395, 2004

0301-5629/04/$–see front matter

ELSEVIER

doi:10.1016/j.ultrasmedbio.2003.11.008

● *Original Contribution*

COMPLEX TIBIAL FRACTURE OUTCOMES FOLLOWING TREATMENT WITH LOW-INTENSITY PULSED ULTRASOUND

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(*Received* 22 *April* 2003; *revised* 10 *November* 2003; *in final form* 18 *November* 2003)

Abstract—A clinical study was conducted to investigate the effect of low-intensity pulsed ultrasound (US) stimulation (LIPUS) on the healing of complex tibial fractures. Thirty complex tibial fractures were randomly assigned to the treatment with LIPUS ($n = 16$) or by a dummy machine (sham-exposed: $n = 14$). The fractures were immobilized by either internal or external fixations according to the clinical indications. LIPUS was given 20 min/day for 90 days. Fracture healing was monitored by clinical, radiological, densitometric and biochemical assessments. The LIPUS-treated group showed statistically significantly better healing, as demonstrated by all assessments. Complications were minimal in the LIPUS group. There were two cases of delayed union, with one in each group. There were two cases of infection in the control group. The delayed-union cases were subsequently treated by LIPUS and the infection cases were treated with standard protocol. Fracture healing in these patients was again treated by LIPUS. (E-mail: ksleung@cuhk.edu.hk)

Key Words: **Low-intensity pulsed ultrasound, Complex tibial fracture, Bone mineral content, Densitometry, Alkaline phosphatase.**

INTRODUCTION AND LITERATURE

Low-intensity pulsed ultrasound (LIPUS) is a fracture-enhancement technique that has been shown to improve fracture healing in acute fractures (Busse et al. 2002; Cook et al. 1997; Duarte 1983; Hadjiargyrou et al. 1998; Heckman et al. 1994; Kristiansen et al. 1997; Nolte et al. 2001; Pilla et al. 1990; Tsai et al. 1992; Xavier and Duarte 1983). Most of the clinical studies focused on simple and closed fractures. There were, however, very few studies on fractures with potential complications (*i.e.,* open fractures and high-energy-induced closed fractures) (Cook et al. 1997; Heckman et al. 1994), in which only grade I open fractures were studied. These fractures are known to have problems of healing due to the high-energy trauma to soft tissues around the fracture sites and on the osseous tissues. Together with the reported clinical evidence on closed fractures, our study in the laboratory also confirmed the positive stimulatory effect of LIPUS on human periosteal cell culture (Leung et al. 2004) in cellular differentiation and functional activation of bone formation, which is vital for initiating and sustaining the cascade of biologic activities in fracture healing (Ozaki et al. 2000; Shedden et al. 1976; Zhang et al. 1996).

In trying to investigate the effect of LIPUS on fracture healing in more complicated fractures with less favourable healing conditions, we conducted a clinical study on the effect of LIPUS on complex tibial fractures. These include open fractures of different grades of severity, comminuted fractures without open wounds and segmental fractures. Our hypothesis is that LIPUS can enhance and accelerate the healing of high-energy tibial fractures.

MATERIALS AND METHODS

Patients recruitment

From September 1999 through April 2002, patients with open tibial fractures and high-energy-induced complex tibial fractures that included comminuted and segmental fractures were recruited in the study. The inclusion criteria were all comminution and open fractures at the tibial shaft, and the exclusion criteria were simple

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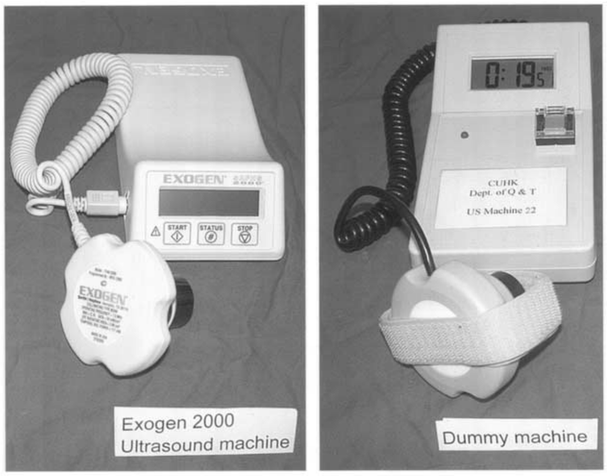

(a) (b)

Fig. 1. US devices used in this study. (a) Exogen 2000® device (Smith & Nephew, Inc.) for treatment group. The US signal is of 1.5-MHz frequency, 1-kHz repetition rate, 200-μs pulse duration and 30-mW spatial-average-temporal-average (SATA) intensity. (b) A dummy machine for control group. The equipment has a digital clock that stops automatically at 20 min, with red light flashing from the LED.

fractures or fractures at other sites. Written consent was obtained from all patients before the start of treatment. The study protocol was approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong (ref. CRE-028). All tibial fractures were fixed with either reamed intramedullary locked nails or external fixator, depending on the fracture patterns and the soft tissue conditions, according to standard protocol of fracture treatment. All Gustilo I and II open fractures and closed diaphyseal fractures were treated by reamed intramedullary locked Grosse and Kempf nails (Stryker Trauma GmbH, Kiel, Germany). External fixators were used for Gustilo IIIA and metaphyseal fractures that could not be fixed by intramedullary nails. All open fractures were treated with emergency debridement and delayed wound closure. Postoperatively, the patients were then assigned to the LIPUS treatment group or to the control group, according to the sequence of admission.

Ultrasound treatment

Patients in the LIPUS treatment group were provided with Exogen 2000® device (Exogen, Smith & Nephew Inc. West Calwell, NJ) (Fig. 1a), and those of the control group were given a dummy machine with the same instruction of application (Fig. 1b). The US signal was of 1.5-MHz frequency, 1-kHz repetition rate, 200-μs pulse duration and 30-mW spatial-average-temporal-average (SATA) intensity. The application of the LIPUS started postoperatively when the patient's condition was stabilized and the open wound was covered either with simple closure or skin grafts. The US probe was applied directly on the fracture site from the anteromedial surface of the tibia. Before the treatment, the emission of US was confirmed with a simple testing device that turned the LED on at the presence of US when in contact with the probe, with a thin layer of coupling gel. The treatment was given with 20 min/day for 90 days because the fracture healing time usually lasted for 12–14 weeks. The patients learned the procedures of application according to the instructions given by the same technician, and continued the application after discharge from the hospital. Coupling gel was used to ensure effective transfer of the acoustic wave to the tissue. The use of the US machine was checked each time when the patients came back for follow-up in the Trauma Clinic by the same technician.

The patients were examined regularly in the Trauma Clinic until the fracture healed. The progress of fracture healing was assessed by clinical examination, standard AP and lateral x-ray films, bone mineral measurement with the dual-energy x-ray absorptiometry machine and plasma bone-specific alkaline phosphatase activities. To achieve the double-blinding, LIPUS treatments and clinical assessments were conducted separately by a research technician and clinicians, respectively, to minimize the subjective interpretation of clinical outcomes.

Clinical examination

Clinical examination was done by a clinician who had no information on the US treatment received by the patients. The assessments were done with respect to fracture site tenderness, the time to start partial weight bearing and full weight bearing. For patients with the fractures treated by external fixator, the removal of the external fixator was determined by the clinical assessment and radiological appearance of callus, without knowledge of the US treatment. The time of external fixator removal was compared between the two groups.

Radiological assessment

The plain x-ray films were taken immediately after fixation, then every 3 weeks for the first 3 months, every 6 weeks for the following 3 months and every 8 weeks for the next 6 months. The films were assessed by three surgeons independently, to note the time of the appearance of the first bridging callus in either the AP or lateral film, the second bridging callus and the third callus.

Bone mineral content measurement

Bone mineral content was measured with Norland DXA machine (XR36; Norland Medical Systems Inc. White Plains, NY) in a well-defined region around the

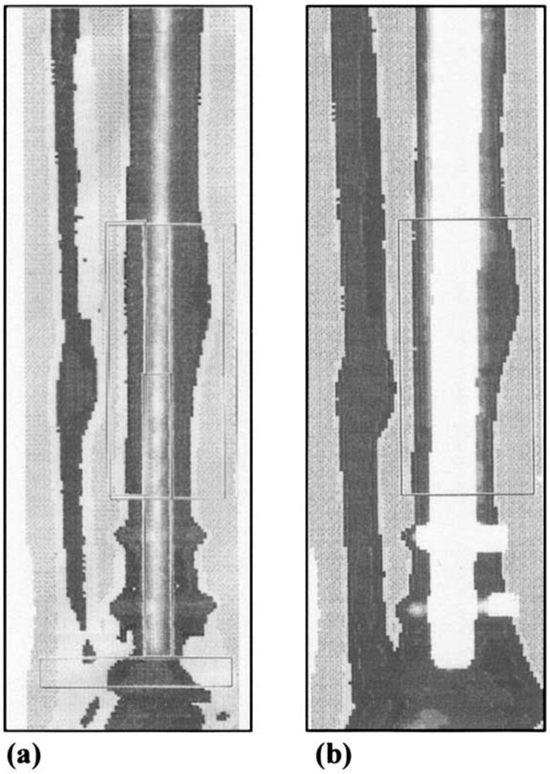

Fig. 2. Fractures fixed with intramedullary nail were measured with "high density analysis mode" of Norland DXA machine. (a) DXA image with normal analysis mode, (b) DXA image with "high density analysis mode", in which scanned points higher than 3.75 g/cm^2 were excluded from analysis calculations.

fracture site. To ensure that the same site was measured each time, external references were made in accordance to the site of the distal locking bolt in the cases with intramedullary locked nail fixation. In the case of external fixator, the reference was made with the transfixing pins. The measurements were done every 3 weeks until 30 weeks after the initial treatment. Fractures fixed with intramedullary nail were measured with "high density analysis mode" (Fig. 2), in which scanned points higher than 3.75 g/cm^2 were excluded from analytic calculations. "High-density analysis mode" is specially designed to exclude large high-density artefacts or prosthetic devices. The percentage change of the bone mineral content compared with the baseline (first measurement after the operation) was calculated.

Plasma bone-specific alkaline phosphatase (ALP) activity

A total of 4 mL venous blood was taken and collected in a heparinized bottle at each follow-up. Plasma was separated by centrifugation at 3000 rpm for 10 min at 4°C. The aliquoted Plasma was stored at −80°C for later analysis. Bone-specific alkaline phosphatase activity (ALP) was measured by the wheat germ lectin precipitation method (Leung et al. 1993, 1995). The percentage change of ALP activity compared with the baseline was obtained.

Statistical methods

The end point of this study was a healed fracture, based on clinical examination and radiographic assessment. The time to clinical healing was defined as the time of starting full weight bearing, at which the fracture was stable as judged by the investigator and the patient did not feel pain on full weight bearing. Radiographically, the appearance of third callus was the sign of fracture healing. The null hypothesis was that the fracture healing time treated with the LIPUS machine was the same as that treated with the dummy machine, and the alternate hypothesis was that the healing time between the two groups was different.

A sample size of $n = 15$ was estimated to achieve a statistical power of 0.8. This calculation was based on a previous similar study (Heckman et al. 1994), with an expectation of 38% decrease in the time to overall healing after LIPUS treatment, on the basis of clinical and radiographic criteria. The data were analyzed with SPSS version 10.0 software (SPSS Inc., Chicago, IL). Student's *t*-test (two-sided) was used to compare the differences between the two groups on the clinical and radiological assessments. The Mann–Whitney *U*-test was used to compare the difference between the treatment group and the control group at different time points for bone mineral content and ALP analysis; *p* values less than 0.05 were considered to be significant.

For radiological assessment, the average time of the appearance of callus scored by three clinicians was averaged. The consistency of the three ratings was determined by intraclass correlation analysis.

RESULTS

Patient information

A total of 28 patients, 25 men and 3 women, with ages ranging from 22 to 61 years old (average 35.3 years) were recruited in this study. Of these, 4 patients had segmental fractures and, thus, a total of 30 fractures were studied. The fracture patterns are shown in Table 1.

The distribution of the LIPUS-treated and the control groups is shown in Table 2; 15 patients (16 fractures) were treated by LIPUS, and 13 (14 fractures) were treated with a dummy machine. The methods of surgical treatment are also shown in Table 2.

All patients managed very well with daily application of LIPUS and the machine recorded the treatment dosage automatically.

Table 1. The fracture characteristics

Fractures	n
Site (4 segmental fractures)	
Proximal	4
Diaphyseal	20
Distal	8
Fracture types	
Closed	13
Open	
I	5
II	7
IIIa	5

Clinical examination

Clinical examination (Table 3) showed that the average time to the disappearance of tenderness at the fracture site in the treatment group was 6.1 weeks and, for the control group, was 7.9 weeks. The difference was statistically significant ($p < 0.05$).

The times of starting partial weight bearing and full weight bearing for the treatment group were 3.8 weeks and 9.3 weeks, respectively. For the control group, the times of starting partial weight bearing and full weight-bearing walking were 4.1 weeks and 15.5 weeks, respectively. The difference in the times to start full weight-bearing walking was significantly earlier in the treatment group ($p < 0.05$).

The time to remove the external fixator for the treatment group was 9.9 weeks and that for the control group was 17.1 week. The difference is also statistically significant ($p < 0.05$).

Radiological assessment

Radiological assessment of the appearance of callus is shown in Fig. 3. Table 4 shows that the time of appearance of all the calli was earlier in the treatment group compared with that of control ($p < 0.05$). The average measured intraclass correlation was 0.6448. The consistency of the ratings by the three clinicians was good.

Bone mineral content measurement

The percentage change in bone mineral content (BMC) at the fracture sites (Fig. 4) showed a higher rate of BMC acquisition in the treatment group from week 6 to week 27, compared with the control. Significant differences were found at weeks 6, 15, 18 and 21 ($p < 0.05$ for all).

Table 2. Surgical treatments in control and treatment groups

	Control	Treatment
Open fracture	8	9
Closed fracture	6	7
External fixator	9	10
Intramedullary nail	5	6

Fig. 3. Prominent callus, often palpable and observable in patient treated with LIPUS. (a) Clinical photograph of a type II open tibial fracture fixed with intramedullary locked nail. Callus was observable (arrow) and palpable 8 weeks postoperation, (b) x-ray film showing the fracture with callus at the same time.

Plasma bone-specific alkaline phosphatase (ALP) activity

Percentage changes in plasma bone-specific ALP activity are shown in Fig. 5. It showed that the ALP

Table 3. Results of clinical assessments (mean ± SD)

	Control (weeks)	Treatment (week)	p value
Disappearance of tenderness at fracture site	7.9 ± 3.5	6.1 ± 2.1	< 0.05
Time to start weight bearing			
Partial	4.1 ± 2.8	3.8 ± 2.2	> 0.05
Full	15.5 ± 3.0	9.3 ± 2.1	< 0.05
Removal of external fixator	17.1 ± 8.5	9.9 ± 3.8	< 0.05

The figures denote the period of time after initial treatment. Statistical analysis was done with the Students t-test. A p value less than 0.05 was taken to be significant.

Table 4. Average time to the appearance of first, second, third callus and remodeling assessed by three clinicians (mean ± SD)

Callus	Average time of callus observed: Control (weeks)	Treatment (weeks)	p value
First callus	9.5 ± 2.2	6.5 ± 1.8	< 0.05
Second callus	12.5 ± 2.9	8.5 ± 2.1	< 0.05
Third callus	20 ± 4.4	11.5 ± 3.0	< 0.05

Statistical analysis was done by the Students *t*-test. A p value less than 0.05 was taken to be significant. The consistency of the ratings among the three clinicians was good (average measured intraclass correlation = 0.6448).

activities were higher in the treatment group from week 6 to week 27. Significant differences were found at weeks 12, 18 and 27 ($p < 0.05$ for all).

Complications

All fractures except one healed in the treatment group. One delayed-union fracture from a closed distal tibial fracture treated by external fixation was subsequently managed with functional bracing, and the fracture healed in a further 2 months with LIPUS treatment. Four patients complained of mild swelling and erythema on the application site after treatment with LIPUS for 2 weeks. The symptoms disappeared after reassurance and the treatment was not interrupted.

There was 1 case of delayed union and 2 of infection in the control group; 2 delayed nonunions were subsequently treated with LIPUS after the external fixator was removed and the fractures were immobilized with a functional brace. Fractures healed after a further 2 months of LIPUS treatment. One infected fracture after intramedullary fixation of a type II open fracture was treated with repeated intramedullary reaming for the debridement and with LIPUS for another 3 months. The fracture healed after 1 year. The other type IIIa fracture initially treated with an external fixator was managed with repeated debridement and LIPUS treatment. The fracture healed after 9 months.

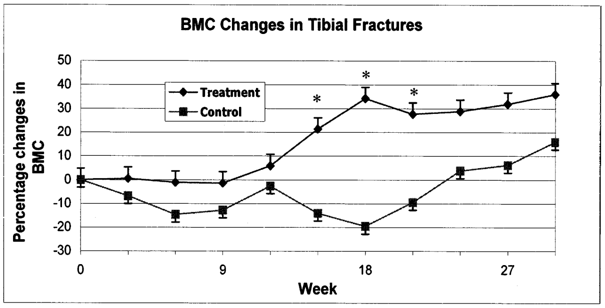

Fig. 4. Percentage change in bone mineral content at the fracture sites. Higher rate of BMC acquisition in the treatment group from week 6 to week 27 compared with the control (sham-exposed), with significant differences at weeks 6, 15, 18 and 21, denoted with * ($p < 0.05$ for all). The error bars represent the standard error of means.

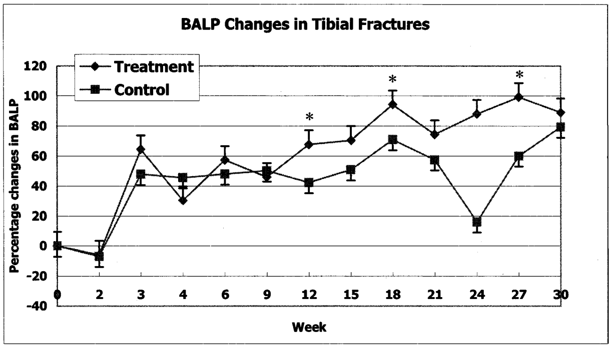

Fig. 5. Percentage change in plasma bone-specific alkaline phosphatase (BALP) activity. BALP activities were higher in the treatment group from week 6 to week 27. Significant differences were found at weeks 12, 18 and 27, denoted with * ($p < 0.05$ for all). Error bars represent standard error of means.

DISCUSSION AND SUMMARY

The results of this clinical study show that LIPUS enhances fracture healing in open fractures and high-energy-induced tibial fractures. The study was well controlled, in which the patients could not distinguish the difference between the US machines and the dummy machines. The clinical examination and the radiological assessment were also done by clinicians without knowing the application of US on the patients. The surgical treatment of the fractures was done according to the clinical conditions and did not affect the selection of LIPUS treatment because the randomization was only done after the surgical operation.

Clinically, the patients treated with LIPUS started full weight-bearing walking earlier and the tenderness in the fracture site disappeared earlier. The appearance of the callus was also earlier for treatment group, and many of the patients with LIPUS treatment showed considerable subcutaneous palpable mass (Fig. 3a). Radiologically, the callus was clearly visible (Fig. 3b). This finding further supports the positive effect of LIPUS on fracture healing. In this series of high-energy-induced fractures, the extent of soft tissue injuries and the comminutions in the fracture sites all indicate that vascular supply in the fracture sites is compromised. Because one of the effects of LIPUS on the cells that take part in fracture healing is

Fig. 6. X-ray films of fractures treated with LIPUS showing callus (arrows) on the posterolateral surface of the tibiae. US probe was applied on the anteromedial surface.

the stimulation of vascular endothelium growth factor (VEGF) (Leung et al. 2004; Street et al. 2000, 2002), the enhancement effect of LIPUS in these conditions may play a vital role in inducing more new vessel growth in the fracture sites and, thus, may improve the vascular supply. It, thus, has a stimulatory effect on both the soft tissue and osseous tissue in the repair process. Because the healing of soft tissue in open and complex fractures determines the ultimate clinical results in managing these difficult fractures, this study further confirms the beneficial effect of LIPUS on fracture healing in less favorable conditions.

The fracture callus not only appeared on the anteromedial surface of the tibia, but also formed on the posterolateral surface of the fracture site, where there was less likely to be a direct effect from the US (Fig. 6). This showed that the stimulatory effect of US was not localized. As demonstrated by our previous *in vitro* results (Leung et al. 2004), the stimulation may be on the bone-forming cells (*i.e.*, the periosteal cells that secrete humoral growth factors) (Shedden et al. 1976; Zhang et al. 1996), which diffuse into the surrounding tissues and stimulate bone-forming cells to enhance the fracture healing. The other possible mechanism of the stimulatory effect is due to the streaming of the ultrasonic waves that skirt around the tibial shaft and focused the effect at the posterolateral surface of the tibia (Hadjiargyrou et al. 1998). High-energy trauma causes severe soft tissue damage, in which significant quantities of bulk fluid may favor the US streaming. However, according to NCRP (2002), the specific mechanism has not been identified yet and this phenomenon needs further proof by direct measurement of the ultrasonic energy at this region. This effect also explains the much earlier appearance of callus around the fracture sites in the treatment group, as compared with the control group.

The enhancing effect of LIPUS on fracture healing is further supported by the significantly higher plasma bone-specific ALP (BALP) activity in patients treated with LIPUS. BALP had been shown to be a good biochemical marker of osteoblastic activity (Leung et al. 1993, 1995). Significantly higher activities were found at weeks 12, 18 and 27. The level remained persistently higher in the later phase of the fracture healing, indicating that the stimulatory effect lasted much longer after LIPUS treatment. The effect may be on the enhancement of cell differentiation of periosteal cells into bone-forming cell lineage, as well as bone-forming activity of these differentiated cells. The sustained increase in the BALP to the later phase of fracture healing may indicate that the number of bone-forming cells is increased by LIPUS treatment in the early phase and that the activity lasts much longer in the treatment group.

The stimulatory effect of LIPUS on fracture healing is also shown in the study of bone mineral content acquisition during fracture healing. In the reported studies that involved monitoring of the change in bone mineral content (BMC) during fracture healing, it is a common finding that the BMC around the fracture site decreases gradually until callus ossification (Eyres and Kanis 1995; Findlay et al. 2002; Leung and Cheung 2002) and callus remodeling. BMC gradually returns to a normal level after normal weight-bearing walking resumes. BMC measurements had been shown to correlate with mechanical stability of healing bones (Augat et al. 1997; Cattermole et al. 1996). In the present study, it was shown that the BMC in the treatment group was persistently higher than that in the control group. The drop in BMC in the treatment group was brief and the magnitude was also small. The BMC increased rapidly and remained persistently high until 23 weeks, when remodeling of the callus commenced. This finding agrees with the clinical and radiological findings and biochemical results in this study, where the treatment group was shown to have a much larger callus in an earlier period during fracture healing.

There was a very minimal complication in the treatment group and the difference in complications between the two groups was statistically not significant. There was almost no complication in wound and soft tissue healing in the treatment group. This may be explained by the healing enhancement of LIPUS in skin wound and ulcers (Reher et al. 1999; Young and Dyson 1990). The beneficial effect of LIPUS on these complex fractures was further proven by the fact that the complications encountered in the control group were also managed with LIPUS subsequently with success.

To summarize, this is a clinical study on a group of well-defined fractures that shows that LIPUS enhances healing in fractures caused by high-energy trauma. LIPUS should, therefore, be recommended in fractures with poor healing potential.

Acknowledgments—Low-intensity pulsed US machines were sponsored by Exogen, Smith & Nephew, Inc. This study was supported by the earmarked grant from the Hong Kong Research Grant Council (RGC reference no: CUHK 4257/99M).

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