



Low Intensity Pulse Ultrasound: A New Tool in the Conservative Management of Diabetic Foot Complicated by Osteomyelitis?

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Abstract

At least 60% of diabetic foot ulcers are complicated by an infection that represents the main cause for major lower limb amputation in these patients. When osteomyelitis is present, surgical treatments and a long antibiotic therapy are required in order to ensure a complete healing, even if these therapies present with limitations due to deficiency in foot perfusion, renal failure and patient's general conditions. Since LIPUS (Low Intensity Pulsed Ultrasound) is able to apply a mechanical stress to the bone, promoting bone formation and is not associated to any known potential adverse sequelae in this study we aimed to try the LIPUS in patients affected by non healing DFO in order to demonstrate a beneficial effect on clinical and radiological healing. Fifteen patients with at least six months non-healing DFO (Diabetic Foot Osteomyelitis) already treated according to standard therapies have been consecutively enrolled. Patients have been submitted to 20 min daily treatment at 30 mW/cm² for 90 days.

All but one patient reached a complete wound healing at the end of the treatment with a corresponding positive impact on bone healing. In the remaining cases a clinical improvement of ulcer was observed.

If confirmed by larger studies, our results demonstrated that LIPUS may be able to accelerate the ulcer and the bone healing and may, then, be considered a low-impact, safe and easy-to-use adjuvant therapy in the armamentarium of DFO.

Keywords: Osteomyelitis; Diabetic foot; Low intensity pulse ultrasound

Introduction

At least 60% of diabetic foot ulcers are complicated by an infection that represents the main cause for major lower limb amputation in these patients. DFO may be present in the 20% of diabetic foot infections seen in outpatient setting, but can involve more than 70% of patients hospitalized for foot infection. These cases are associated with worse outcomes compared with soft tissue infections [1].

The epidemiology of osteomyelitis is related to the one found in soft tissue and is often polymicrobial, involving Gram+ germs, such as *S. aureus*, *Streptococci* and *Enterobacteriaceae* and less commonly Gram negative, (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* and *Pseudomonas aeruginosa*). Bacteria may gain access to the bone by contiguous spread, entering from overlying soft tissue and penetrating the cortex and, then, the bone marrow [2].

Focal osteopenia, periosteal reaction, and cortical erosion, usually evident in the radiographs 10-15 days after the beginning of bone involvement and a confluent intramedullary pattern of decreased signal intensity on MRI T1-weighted images may be generally considered the most reliable signs of osteomyelitis, even if examination of a bone sample, with microbiological or histopathologic methods, is the gold standard for the diagnosis [3].

A conservative approach, exclusively based on long antibiotic therapy or surgical removing as little bone and soft tissue, is usually required in order to ensure a complete healing, even if these therapies present with limitations in many patients due to deficiency in foot perfusion, renal failure and patient's general conditions [4,5].

For over 20 years, patients have used (LIPUS) as an adjunct treatment to improve bone healing in different clinical settings. In particular, many patients presenting with fractures or

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osteotomies demonstrated a decrease in days to full weight bearing, pain, and radiographic healing, even if a large variability between studies has been observed [6]. Although the exact underlying mechanisms remains unclear, many papers have suggested that LIPUS can accelerate bone healing in many ways, such as stimulation of osteogenic gene expression, microcirculation and angiogenesis [7,8].

Since LIPUS is able to apply a mechanical stress to the bone, promoting bone formation and is not associated to any known potential adverse sequelae, we report a unique series of diabetic patients treated with LIPUS as adjuvant treatment in a conservative approach to osteomyelitis.

Materials and Methods

Recruitment

From June 2016 to December 2016, 15 patients referring to the Difficult Wounds Center affected by Diabetic Foot Osteomyelitis (DFO) were consecutively enrolled. The diagnosis of DFO was established on the basis of a combination of probe-to-bone test and plain X-ray, as previously published. The probe-to-bone test was performed using a metal forceps and the result was considered positive when a hard or gritty surface was felt by the physician. X-rays were considered "positive" for osteomyelitis if they showed lytic lesions, endosteal scalloping, loss of trabecular architecture, cortical disruption, periosteal elevation, a sequestrum or involucrum. Inclusion criteria were as follows: Age \geq 18 years; type 2 diabetes neuropathic ulcers complicated by osteomyelitis, non-healing for at least 6 months, even if treated according to the standard therapy (long-term antibiotic therapy, best wound healing therapies, soft tissues debridement and offloading), compliance to attend the visits during the follow-up period. The wounds were all classified as Texas III B.

Exclusion criteria were as follows: patients with severe infections according to Infectious Diseases Society of America classification; necrotizing soft tissue infections; limb ischemia; Charcot foot; glycated hemoglobin \geq 10% (86 mmol/mol); pregnancy; antibiotic allergies and patients with oncologic diseases and a life expectancy less than 6 months.

Outcome measures

At the enrollment all patients had a complete evaluation that included a medical history, a focused physical examination and anteroposterior and lateral radiographs. Microbiological specimen after soft tissues debridement was obtained in all cases. Magnetic Resonance Imaging (RMI) was performed on a case-by-case basis. Radiographs scans were repeated 12 weeks after the initiation of LIPUS. The follow up period was extended for all the patients up to 6 months after the end of treatment. Inflammatory markers were measured in blood samples at the beginning of the study (day 0) and 12 weeks after the initiation of LIPUS. Inflammatory markers were defined as follows: leukocytosis was defined as a white blood cell count $>10 \times 10^3$ uL; elevated Erythrocyte Sedimentation Rate (ESR) was defined as an ESR >20 mm/h; and elevated C-Reactive Protein (CRP) was defined as a CRP level >5 mg/L.

The neurological examination was undertaken using Semmes-Weinstein 4.56 monofilaments (Epitech group, Italy), biotensimeter (VPT3, Sei mgrs.r.l.) and electromyography. Peripheral arterial disease was diagnosed on the basis of ankle brachial index \leq 0.9 and a color Doppler ultrasound performed by a vascular surgeon.

Moreover, a transcutaneous oxygen tension was determined using a Clark-type oxygen-sensing electrode (TMC 400, De Mori, Italy). The electrode was attached to the dorsum of the foot at the base of the second metatarsal, and the controlled heating element around the electrode was warmed to 44°C. Measurements were performed with the patient resting in supine position, covered by a light blanket, and at a room temperature of 21°C to 23°C. The instrument was calibrated against a known standard before each use and the probe was left in place for 20 min before a final reading was obtained.

Antibiotic treatment was initially empiric, according to the clinician's preference, and consisted of the following three regimens: ciprofloxacin 500 mg b.i.d.; levofloxacin 500 mg q.d.; or clindamycin 300 mg t.i.d. The antibiotic regimen was modified according to the results of the antibiogram. On the basis of this characterization, a targeted systemic antibiotic therapy was performed for a period of 8 weeks. Only at the end of this period in case of worsening or not a clear improvement in ulcer healing and after critical re-evaluation of all clinical features, patients were judged eligible for LIPUS.

During the treatment patients were followed weekly and dressed according to international guidelines. Off loading was performed in all cases, according to international guidelines [9].

Healing was defined as the complete epithelialization of the ulcer and/or the surgical wound that was created while treating the infection. Time to healing was defined as the time in weeks from the date on which osteomyelitis was diagnosed to the date of healing. Recurrence of osteomyelitis was defined as the appearance of bone infection at the same or an adjacent site after healing.

Lipus treatment

Treatment with the LIPUS device (Osteotron IV, Fisionoleggio, Italy) was applied at home from the patient for 20 min once daily on the site of bone affected by osteomyelitis after marking the position under fluoroscopic guidance. The LIPUS device had an output frequency of 1.5 MHz, 750 kHz an output power of 30, 45, 60 mW/cm². The intensity of treatment was chosen on the basis of osteomyelitic bone (superficial or deep position). The treatment was performed for 90 days.

Results and Discussion

Fifteen patients (8 males; 7 females) were followed up for at least 12 months. The main features of patients are resumed in Table 1. Wound's area was measured at the enrollment and resulted in a median value of value of 0.9 cm². Ulcer location is described in Table 2. The duration of the ulcers expressed as median was 27 weeks. All but two ulcers were positive to PTB test and an increase of serum inflammatory markers (C-reactive protein and erythrocyte sedimentation rate) between 1.5 and 2 times compared to normal values was observed in 90% of patients (Table 3). The frequency of isolated pathogens was 53%, 27%, 10% and 10% for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterobacter cloacae* respectively. Empiric antibiotic treatment was: levofloxacin 500 mg q.d. in 60% of patients; ciprofloxacin 500 mg b.i.d. in 30%; and clindamycin 300 mg t.i.d. in 10%. After culture results, antibiotic therapy was modified according to the antibiogram in seven patients as follows: teicoplanin 200 mg q.d. in five cases; trimethoprim 160 mg/sulfamethoxazole 800 mg b.i.d. in 2 patients (8.4%). No side effects of these therapies were observed. LIPUS treatment was performed in all patients, as previously described. No side effects were recorded. No minor amputations have been performed in these patients. The



Figure 1: Ulcer presenting with I metatarsal head osteomyelitis; a) before treatment; b) 3 months after the LIPUS treatment; c) X-ray at the beginning of the study; d) X-ray after LIPUS treatment.



Figure 2: Ulcer presenting with V metatarsal head osteomyelitis; a) before treatment; b) 3 months after the LIPUS treatment; c) X-ray at the beginning of the study; d) X-ray after LIPUS treatment.

only surgical treatment was a debridement performed in ambulatory setting. The wound bed cleansing was performed by using a solution of polyhexanide and the wound dressing with sodium chloride impregnated gauzes.

Infected areas typically appeared at X-rays dark, with periosteal thickening, lytic lesions, endosteal scalloping, osteopenia and loss of trabecular architecture. When performed, MRI showed low signal intensity on T1-weighted sequences and high signal intensity on T2-

Table 1: Demographic and clinical characteristics of the study population.

Variables	15 patients
Female sex	6 (40)
Age	68 (51-92)
BMI (kg/cm ²)	27 (25-30)
Diabetes therapy	
OHA	3 (20)
OHA + insulin	4 (26.6)
Insulin	8 (53.3)
Glycemia (mg/dL)	148 (115-220)
HbA1c (mmol/mol)	7.1 (5.9-8.7)
Smoking	5 (33.3)
Alcohol abuse	-
Hypertension	12 (80)
High cholesterol level	9 (60)
Coronary hear disease	5 (33.3)
Retinopathy	6 (40)
Nephropathy	8 (53.3)
Previous ulcer	9 (60)
Previous amputation	7 (46.6)
S-W monofilament	13 (86.6)
Vibration threshold	13 (86.6)
Ankle-brachial index	1.3 (1.05-1.4)
tcPO2 (mmHg)	42 (35-46)

Abbreviations: OHA: Oral Hypoglycemic Agents; S-W: Semmes-Weinstein; tcPO2: Transcutaneous Oxygen Pressure. Data are given as n (%) or median

Table 2: Ulcer location.

Location	
rearfoot	2 (13.3)
midfoot	2 (13.3)
forefoot	
I ray	7 (46.6)
rays other than I	4 (26.6)

Data are given as n (%)

weighted sequences, demonstrating changes in the bone marrow. After 3 months of LIPUS radiological criteria of bone healing include: well-organized consolidation of periosteum, reduction of bone lucency, reduction of pathological fractures related to bone infection.

The levels of inflammatory markers (ESR and CRP) measured on day 0 were elevated in 90% of patients, but no one presented with leukocytosis. Twelve weeks after the initiation of LIPUS a normalization of these markers was observed in all patients.

All patients healed after LIPUS treatment, except two (Figure 1 and 2). In one the healing was observed after 2 months from the end of the LIPUS treatment and in another one an improvement was observed in the ulcer area, without a complete reepithelization. During the period of follow-up after healing (1 year) no recurrence was found (defined as absence of any sign of infection at the initial or a contiguous site).

As far as we know the current observational study is the first attempt to try the LIPUS treatment in the conservative management of DFO. Previous studies dealing with this field, clearly demonstrated

that the first approach is an antibiotic therapy combined or not with conservative surgery (excision of infected bone and non viable tissues) [10]. In a prospective randomized clinical study the use of prolonged antibiotic therapy without surgery has been compared to conservative surgery and demonstrated a similar rate of wound healing and healing time [5]. On the other hand, an aggressive surgical approach based on minor amputation may be mandatory in some circumstances. In any case, the best approach to DFO is still a hot topic and should take into account the general conditions of these fragile patients. The main problem is that also a prolonged antibiotic therapy alone is associated to risks of side effects, infection recurrence, and bacterial resistance and may represent a huge problem in these kinds of patients, often presenting with renal failure and/or critical general conditions. Moreover, the optimal duration of antibiotic therapy is not completely defined, ranging in the guidelines from three months in case of antibiotic therapy alone, 4-6 weeks when the infected bone is not completely removed, to one week if completely removed [11]. Moreover, the critical general conditions may render difficult the surgical approach because of the anesthesiological and cardiologic risks. Finally, the surgical treatment can cause the potential alteration of foot architecture. The motor and sensory neuropathy in conjunction with a partial amputation may impair the biomechanics of the foot, promoting re-ulceration or transfer ulceration in the new high-pressure site. The use of PMMA (polymethylmethacrylate) spacer after resection of bone has been tried and is actually considered a viable and noteworthy limb salvage tool when used together with a comprehensive surgical plan. However the description of this method in the literature is limited to few patients [12].

It is more than a decade that LIPUS have been suggested as a possible co-treatment option for fracture healing. Since bone may be considered a piezoelectric material, ultrasound therapy has been used to encourage osteogenesis, accelerate fracture consolidation, and augment bone mass. Heating, cavitation, and acoustic streaming have been suggested as the key physical mechanisms. In an *in vitro* study, Katiyar et al. concluded that a short-term stimulation with optimum intensity can enhance the proliferation of preosteoblast like bone cells that play an important role in bone formation and accelerated fracture healing. LIPUS stimulation may also control the proliferation, differentiation and bone formation of osteoblasts directly by *via* the integrin/FAK/PI3K/Akt and the ERK signaling pathways and indirectly by an amplified secretion of Prostaglandin E2 from osteocytes. And finally ultrasound may upregulates the Nitric-Oxide Synthase (iNOS) expression in osteoblasts by a Hypoxia-Inducible Factor-1 (HIF-1) alpha-dependent mechanism, which involves the activation of Integrin Linked Kinase (ILK)/Akt and mTOR pathways *via* the integrin receptor. On the other hand, other authors revealed that LIPUS treatment can restore the normal osteogenic differentiation of MSCs from disuse by daily short-duration stimulations and may induce the osteogenic differentiation of ADSCs *in vitro*. In a more recent study, in a coculture system, LIPUS treatment significantly increased in the endothelial cells the expression of the proliferation marker Cyclin-D1 and the differentiation markers Osterix, Runx2, and ALP in osteoblasts, indicating that existence of endothelial cell-promoted proliferation and differentiation of osteoblasts [13]. A concomitant clinical study confirms in healthy volunteers that ultrasound may affect micro-circulation, stimulating significant changes in flow, SO₂ and rHb levels [7].

Even if on the bases of the literature there are no conclusive

evidences on the use of LIPUS on fresh fractures, the applicability to other types of fracture or osteotomy is still open to debate. This is why we tried the ultrasound on these fragile patients in order to add a non invasive tool in the armamentarium of DFO.

In our patients LIPUS treatment demonstrated to be able, in association to medication, off loading and antibiotic therapy, to heal all ulcers, except two. We hypothesize that this treatment by helping bone consolidation and micro-circulation in the wound area may accelerate wound healing and promote the bone reaction, avoiding also recurrences. In the patient, who acquired healing after two months from the end of the LIPUS treatment, we believed that the long lasting presence of wound and osteomyelitis (more than nine months) may explain the more difficult healing process. Finally, recording data of the patient who presented a local improvement at the beginning, but did not acquire a complete reepithelialization, we hypothesized that the concomitant heart failure during the first month of therapy may explain the result. The pitting edema involving also the wound area could alter the transmission of ultrasound, undermining the efficacy.

To the best of our knowledge, this is the first report showing that LIPUS might be a feasible treatment for DFO. If confirmed in a larger series, our results may identify LIPUS as a new tool in the armamentarium of conservative treatment for this difficult clinical challenge.

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