REVIEW ARTICLE

Therapeutic Applications of Low-intensity Pulsed Ultrasound in Osteoporosis

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Abstract

Ultrasound (US) waves due to their unique features can be a treatment option for osteoporosis. Initial studies have shown promising but controversial outcomes. Several preclinical and clinical studies have been conducted on osteoporosis and studies are ongoing to find optimum US parameters, mechanisms of action, and therapeutic efficacies of these techniques for osteoporosis treatment. This paper was aimed to review the recent advances of using US waves in the treatment of osteoporosis and possible mechanisms of actions. The databases of PubMed (1980-2016), EMBASE (1980-2016), Web of Sciences (1980-2016), and Google Scholar (1980-2016) were searched using the set terms. The obtained records were reviewed, and relevant studies were selected for comprehensive review of the current literature. Low-intensity pulsed US (LIPUS) has biological effects on the bone healing process and it can accelerate bone regeneration. Current evidence is limited on the efficacy of US waves for treatment or prevention of osteoporosis; however, the initial studies are promising. The US waves can promote osteoblast and inhibit osteoclast, enhance angiogenesis, trigger expression of different genes associated with osteogenesis. No definite dose-response existed on the clinical trials of US wave applications. The current evidence shows the therapeutic efficacy of US waves particularly LIPUS for osteoporosis treatment; however, to observe therapeutic outcomes long-term US stimulation is required. No definitive dose-response is proposed for osteoporosis. Further *in vitro* and clinical trials should be conducted to develop US-based techniques for the treatment of osteoporosis as a clinical treatment option.

Key words: Mechanism of action, osteoporosis, treatment, ultrasound

INTRODUCTION

Osteoporosis is among the common musculoskeletal disorders worldwide. The first choice of treatment for osteoporosis is currently medication. Bisphosphonates are the most common medications administered for osteoporosis treatment and prevention. The main types of these medications include alendronate (Fosamax), risedronate (Actonel), ibandronate (Boniva), and zoledronic acid (Reclast).[1] Hormones, such as estrogen, and some hormone-like medications such as raloxifene (Evista) are also approved medications for preventing and treating osteoporosis. The medications have different side effects considering the relatively long-term administration of such medications.[2] Moreover, fewer women use estrogen replacement therapy now because it may increase the risk of heart attacks and some types of cancer. Therefore, developing a new non-medication treatment for osteoporosis is necessary.

During the recent years, several non-medication treatments have been developed for the treatment of bone related disorders.[3,4] At present, ultrasound (US) waves are known as therapeutic tools which are widely used in various fields of diagnostic and therapeutic medicine including structural and functional imaging, soft tissue injuries repairing, recovery of musculoskeletal anomalies and injuries, and reducing the pain.[5-7] US is a mechanical longitudinal energy in the form of waves that can transfers mechanical energy into the tissues as a propagating pressure wave.[8,9] Bone regeneration

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involves a complex process such as inflammation, cellular proliferation and differentiation, chemotaxis, synthesis of an extracellular matrix and finally remodeling.[10,11] Although the cellular mechanisms of the US effects are not clearly understood, several *in vitro* and *in vivo* studies have demonstrated that low-intensity pulsed US (LIPUS) has biological effects on the bone-healing process and it can accelerate bone regeneration.[12-15] The results of several histological studies have shown that LIPUS has an important influence on key functional activities of all major cell types involved in bone healing, such as osteoblasts, osteoclasts, chondrocytes, and mesenchymal stem cells.[16,17] In addition, several studies showed a positive effect of US to increase the levels of intracellular calcium incorporation in cultures of differentiating bone and cartilage cells. Increases in calcium incorporation were modulated transforming growth factor (TGF)-beta and adenylate cyclase activity. LIPUS also has been shown to increase the intracellular calcium concentration in chondrocytes and increase the percentage of calcified cartilage. Others have suggested that LIPUS have a stimulatory effect on endochondral ossification. Concurrently, stimulation of endochondral ossification is due to stimulation of bone cell differentiation and calcified matrix production.[18-21] In addition, it has been shown in an animal model that LIPUS leads to stimulation of vascular endothelial growth factor. Others have shown that LIPUS treatment increases the degree of vascularity, indication that US increases blood flow.[22,23] This study reviewed the mechanism of US stimulation in osteoporosis treatment.

LIPUS AND OSTEOPOROSIS

Osteoporosis is the most common bone disorder and a major and growing health problem worldwide. Several risk factors are involved in the occurrence and progression of osteoporosis including aging, sedentary lifestyle and estrogen deficiency due to menopause, ovariectomy, and hormonal therapy.[24-27]

Various medications such as estrogen, bisphosphonates, calcitonin, calcium, and vitamin D have been used in the treatment of osteoporosis for many years. Although they are mainly used therapeutically as bone resorption inhibitors, they have no significant long-term effects.

Numerous *in vivo* animal and clinical trials studies have shown that LIPUS because of its properties and positive effects on the generation and activation of bone cells is capable of accelerating and augmenting the healing of osteoporosis. LIPUS produces the pressure waves, which in duce biochemical and molecular events at the cellular level and whereby accelerated healing of osteoporosis.[22,28-32] The results of biomechanical and histologic investigations prove that LIPUS have effects on bone mineral density and mechanical strength. They also concluded that LIPUS stimulates bone formation in distraction osteogenesis and acceleration of healing or strength.[33,34] Various studies have shown that LIPUS not only prevent bone loss but also restore bone mass. The finding suggested that LIPUS therapy, if scaled for whole body use, has clear clinical benefits for the treatment of osteoporosis.[22,35-37]

LIPUS IN CELLULAR LEVELS

Most of the experimental studies showed that LIPUS treatment influence cell membrane permeability and increase cellular activity.[31,38-40]

Several genes such as alkaline phosphatase (ALP), bone sialoprotein (BSP), collagen type I, osteocalcin (OC), and osteonectin (ON) are characteristic of osteoblast differentiation. They are overexpressed during the process of osteogenesis.[41] The results of a study that evaluated the genetic expression and response to LIPUS in rat osteoblastic cells showed early response genes in bone marrow-derived stromal cells. In this study, the gene expression level in LIPUS group is demonstrated and calculated over the control group. The results between LIPUS stimulated group and sham control group for cyclooxygenase-2, early growth response-1, TGF-beta stimulated clone-22, ON, and osteopontin had shown a statistically significant difference.[42] Another study evaluated the effect of LIPUS on the differentiation of pluripotent mesenchymal cell line C2C12 by examining particular mRNA and protein expression levels. C2C12 cells have the capacity to differentiate into myoblasts, osteoblasts, chondroblasts, or adipocytes. The results determined that LIPUS stimulation increased Runx2 protein expression and phosphorylation of ERK1/2 and p38 mitogen-activated protein kinase (MAPK). They also demonstrated that LIPUS stimulation converts the differentiation pathway of C2C12 cells into the osteoblast and/or chondroblast lineage via activated phosphorylation of ERK1/2 and p38 MAPK.[43] Mukai *et al*. (2005) in their *in vitro* study demonstrated that LIPUS promoted the mRNA expression of type II collagen, type X collagen, aggrecan, and TGF-β in rat chondrocytes.[44] In addition, Chen *et al*. (2003) reported that LIPUS stimulation elevated Runx2 mRNA expression and gradually promoted OC mRNA expression in human osteoblasts.[40] Other several *in vitro* studies had shown LIPUS elevated mRNA levels for insulin-like growth factor-I, OC, and BSP and also it was found to stimulate mRNA expression of the bone matrix proteins ALP and OC in UMR-106 cells.[16,17] In addition, there have been some reports that LIPUS may have a direct effect on cell membrane permeability.[23,45-50]

TEMPERATURE VARIATIONS

The range of energies used in LIPUS treatment is relatively low which is the range of non-thermogenic and nondestructive. High-intensity US waves that are used in therapeutic and surgical applications (1-300 W/cm^2) generates considerable heat in living tissue.

Some of the investigators reported that the therapeutic benefits observed with LIPUS stimulation involve non-thermal mechanisms. Contrary, some researchers believe that the ability of LIPUS to stimulate changes in tissues and cells may be related to the temperature rising effects induced by energy absorption.[51-54] High intensities (1000-3000 mW/cm^2) have temperature effects and can cause considerable heating of tissues. Whereas the heating effect from LIPUS (20 to 50 mW/cm^2) is estimated <11°C,[54] some enzymes such as matrix metalloproteinase or interstitial collagenase have been shown to be very sensitive to small variations in temperature. Therefore, minimal heating effects may affect on them.[55,56] Chang *et al*. (2002) investigated thermal effects of US stimulation on fracture healing. They reported that difference between the microwave hyperthermia treated limbs and the sham-treated limb was not quite statistically significant. They have suggested that LIPUS stimulation could increase the new bone formation but its effects probably are not mediated via hyperthermia.[54]

Other investigators suggested that the therapeutic benefits observed in tissues and cells after LIPUS stimulation may also be associated with nonthermal processes such as acoustic streaming and cavitation. They have suggested that cavitation mechanisms may due to an increase in protein and collagen synthesis observed in human fibroblasts after US stimulation.[57-62]

EFFECTS OF VARIOUS INTENSITIES

To finding an optimal LIPUS protocol studies have examined several different low intensities and frequencies. Several studies have investigated the role of intensity in therapeutic effects and an attempt to determine the optimal LIPUS setting. High energy and intensity US through absorbed by tissues lead to increase tissues temperature and kill malignant cells.[7]

To reduce pain and muscle spasms, to decrease joint stiffness, and to improve muscle mobility use applications of US in intensities of 1-3 W/cm^2.[63]

The results of a study showed that the response of cells to the US is highly dependent on the intensity. With increasing US intensity to 120, 390, and 1490 mW/cm^2 expression of ALP showed progressively increased.[64]

In a study, two different LIPUS intensities compared directly to investigate the relationship between intensity of and restoring the mechanical properties of a rat femora following fracture (50 and 100 mW/cm^2). The results showed that the group was treated with 50 mW/cm^2 LIPUS intensity had significantly greater maximum torque and torsion stiffness compared to 100 mW/cm^2 treated femora and untreated controls.[13]

Another parameter of the US signal that has also been investigated in stimulating bone osteogenesis is the frequency.

Several studies showed that the response of cells to the US is also dependent on the frequency. In a study therapeutic results of two protocols with different frequencies (1.5 MHz, 3.0 MHz) and constant intensity (500 mW/cm^2) on rat fibulae fractures healing were compared. Results showed that protocol with 1.5 MHz frequency had more advanced radiographic and histological healing.[46] Results of another study demonstrated that there was no significant difference in therapeutic results of two protocols with different frequencies (1.5 MHz, 0.5 MHz) and constant intensity (30 mW/cm^2). In this study maximum torque and torsional stiffness was investigated and both frequencies almost equally led to increase these parameters compared to untreated controls.[65] Another two protocols with different frequencies (1.5 MHz, 3.0 MHz) and constant intensity (500 mW/cm^2) were investigated by Tsai *et al.* (1992). Results of the group that had been treated with 1.5 MHz showed significantly greater mineral apposition rates.[66]

Based on the results obtained to stimulate osteogenesis experimentally and clinically frequency of 1.5 MHz has been more commonly used.

TIME-DEPENDENT EFFECT

There have been some reports that LIPUS shows positive effects on the healing of fresh fracture, nonunion and delayed union and also it accelerates bone maturation in distraction osteogenesis in clinical treatment and animals models.[67-72]

On the other hand, there are some controversial results in regard to LIPUS is most effective during the lengthening phase, but the optimal timing of LIPUS has not been established. The researchers sought to determine the stage of fracture repair process that US have the greatest influence effects.

Several *in vivo* experimental studies investigated the effect of LIPUS stimulation on the various phase of fracture healing. Results of some studies suggested that LIPUS does not affect the remodeling phase of fracture healing. These results determined that LIPUS have a significant effect on the earlier inflammatory or accelerate to callus formation phases of healing.[22,31,73-75]

In a study with a rat femoral fracture model, hard callus area, bone mineral content, mechanical torsion properties were measured at 4 different periods (1-8 days, 9-16 days, 17-24 days, 1-24 days) after expose to LIPUS, along with histologic analysis. The findings reported statistically significant increases in all measured parameters in all groups when compared with the control group. They suggested that LIPUS acts on some cellular reactions at each stage of the fracture healing process.[73]

Azuma *et al*. (2001) in an experimental study measured mechanical and histological changes at different time periods during the healing process. They investigated the timing or duration of stimulation effect after 8 and 25 days of LIPUS treatment. They reported that the results had not shown significant effect associated with the timing or duration on the bone mineral content, but they also reported a significant increase in bone stiffness and maximum torque in LIPUS group.[73]

CONCLUSION

Most of the studies showed a positive effect of US on bone healing. Numerous studies have proved the effect of LIPUS on bone regeneration, changes in bone mineral content and density. They have also demonstrated LIPUS increase callus formation, and its biological changes. Based on the results, investigators suggested that LIPUS therapy with smaller and continuous mechanical stress is more useful in preventing bone loss and bone remodeling in a clinical setting.[13,38,40,54,64-66] LIPUS is affected without pain, without the need for hospitalization and it is portable by the patients. Between the methods available to enhance musculoskeletal disorders healing, US has suggested as a safe, practical, and effective treatment.[22,28,34,35] In our opinion, extensive clinical and experimental and long-term studies investigating biophysical mechanisms of LIPUS are required.

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