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Review Article

The role of low-intensity pulsed ultrasound on bone and soft tissue healing

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Abstract: Low-intensity pulsed ultrasound (LIPUS) is a widely used therapy method for bone and soft tissue injury patients. However, the exact mechanism is not very clear until now. In this review, we summarized the possible mechanisms of LIPUS therapy on bone-tendon junction (BTJ), stem cells, muscle injury, wounds, bone healing, osteoporotic fracture, and osteoarthritis. Once the underlying mechanism of LIPUS is clarified, many patients will obtain benefits from this treatment method.

Keywords: Low-intensity pulsed ultrasound (LIPUS), bone-tendon junction (BTJ) osteoporotic fracture, endochondral ossification

Introduction

Ultrasound is widely applied for clinical imaging examination purposes, which is also a kind of physiotherapy to treat many bone and soft tissue diseases. The range of ultrasound intensities for imaging purpose is 0.05-0.5 W/cm^2. The surgical and therapeutic applications of ultrasound intensity range is 0.2-100 W/cm^2 [1]. Low-intensity pulsed ultrasound (LIPUS), at intensity of 30 mW/cm^2, is a widely used, and Food and the Drug Administration (FDA) approved therapy method for promoting bone healing [2, 3]. LIPUS is a noninvasive modality, transmitting mechanical energy transcutaneously into biological tissues [4]. Consumption of the ultrasound generated mechanical energy lies on the attenuation and absorption of the transmitted tissue. LIPUS offers an instant mechanical stimulation in promotion of mineralization, endochondral ossification, and osteoblast proliferation [5-7].

In the process of LUPUS treatment for bone fracture, the application angle also played a key role in the therapy process. Chung SL et al reported that among the angles of 0°, 22°, 35° and 48°, the LUPUS transmission at 35° demonstrated the best clinical effect on fracture healing [8]. The same therapy method which was applied in the different stage of disease might produce different effect for the patients. Therefore, it was important to select appropriate stage of disease to implement LUPUS treatment. Fu SC et al reported that LIPUS increased collagen synthesis via up-regulating mRNA expression level of COL1A1 and COL3A1, and decreased matrix remodeling by down-regulating mRNA expression level of decorin and biglycan, demonstrating that LIPUS ought to be performed in the granulation stage but not in the remodeling stage in order to accelerate tendon healing process [9]. Although LIPUS stimulation at 30 mW/cm^2 is an approved therapy method by FDA for improving healing process in bone fractures and non-unions, Angle SR et al reported that the dose of 2 mW/cm^2 could exert higher mineralization effect on rat bone marrow stromal cells than 30 mW/cm^2 after five days LUPUS therapy, indicating that the most appropriate intensity of LIPUS for bone healing

might be adjusted in the early stage following bone fracture if this result was proved to be true in vivo [3]. As for the mechanism of therapeutic effect of low intensity ultrasound, Man J et al reported that continuous kHz ultrasound accelerated scratch-wound closure via cell migration while pulsed MHz promoted wound closure through both cell proliferation and migration [10].

Thus, we review the related available literature on the role of low-intensity pulsed ultrasound on bone and soft tissue healing, investigating its effect both at in vivo and in vitro level, and summing up the evidence available until now.

LIPUS on bone-tendon junction (BTJ)

After trauma and reconstructive surgeries, bone-tendon junction (BTJ) repair needed a long time immobilization which was related to postoperative weak knee. Lu MH et al reported that low intensity pulsed ultrasound obviously promoted newly formed bone at the BTJ healing interface and up-regulated stiffness of the junction tissues with higher bone-mineral density, indicating that low intensity pulsed ultrasound might be an effective therapy for BTJ injury [11]. LIPUS and/or functional electrical stimulation (FES) treatments significantly increased the area and bone mineral content of new bone. The failure load and ultimate strength of on patella-patellar tendon (PPT) complex were also highly improved in the three treatment groups. More new bone formed and higher tensile properties were showed in the LIPUS + FES group compared with the LIPUS or FES alone groups [12]. Jeremias Júnior SL et al [13] reported that Low-intensity pulsed ultrasound which was transmitted by a conventional ultrasound device could shorten the time for the calcaneus tendon healing process in rats, in accordance with the results of mechanical stress testing. The low-intensity pulsed ultrasound increased bone mineral density and osteoblast activity at the tendon-bone interface, involving in protein expression increase of VEGF, RUNX2 and Smad4, indicating that ultrasound therapy might develop an alternative method to treat patients following reconstructive surgeries involving the tendon-bone interface [14]. The possible molecular mechanism of the repair of acute ruptured Achilles tendon by LIPUS might involve in promotion of COX-2 and EP4 and increase of TGFbeta1 followed by collagen I and III [15].

LIPUS on stem cells

LIPUS promoted osteogenesis of adipose stem cells (ASCs), which were related to mRNA levels up-regulation of transcription factor 2, osteopontin and osterix, as well as protein levels promotion of runt-related transcription factor 2 and osteopontin, indicating that LIPUS promoted osteogenesis of ASCs [16].

LIPUS together with intra-articular injection of Mesenchymal Stem Cells (MSCs) could promote sagittal condylar development in left temporomandibular joint of rat and LIPUS treatment alone might promote sagittal condylar development. However, MSC application alone did not influence sagittal condylar growth [17]. Murine ASC adipogenesis was enhanced via mRNA promotion of PAR-γ1 and APN, as well as protein up-regulation of PPAR-γ, indicating that LIPUS could exert beneficial effects on adipose-derived stem cells [18]. The exogenous mesenchymal stem cells (MSCs) demonstrated an obviously faster remodeling for fracture healing in rat model, which could be enhanced by low intensity pulsed ultrasound (LIPUS). The combination of MSCs and LIPUS might be used to deal with bone delayed union or nonunion [19]. LIPUS simulated the effects of syndecan-4 engagement to advance Rac1 activation and focal adhesion formation to accelerate cell repair process, indicating the possible signal pathway involved in the molecular effect of ultrasound in culture [20]. Kumagai K et al reported that LIPUS promoted the homing of circulating osteogenic progenitors to the site of femoral fracture which might contribute to new bone formation [21].

LIPUS on muscle injury and wounds

LIPUS decreased the wound width and unhealed areas in palatal excisional wounds of rats, indicating that LIPUS was beneficial for epithelial and connective tissue closure [22]. Rennó AC et al reported that both LLLT and US therapies might exert beneficial effects on injured skeletal muscle, but LLLT demonstrated better beneficial effects on muscle metabolism following muscle injury in rats [23]. LIPUS treatment led to higher proliferative rate and number of myoblastic cell, together with increase in myogenin and actin proteins of cells, and myofibers, fast-twitch of gastrocnemius muscle of the left leg in mice, indicating that LIPUS could

promote muscle healing following laceration injury [24].

LIPUS on bone healing

As for the mechanism of clinical effect of ultrasound for bone fracture, low-intensity pulsed ultrasound obviously promoted the rate of bone union and volumetric bone mineral density, but this effect was abolished in sciatic neurectomy rats, indicating that the sensory innervation of bone significantly took part in sensing and responding to ultrasound treatment in rats [25]. LIPUS cooperated with alendronate to significantly promote BMD in aged rats following a unilateral proximal tibial osteotomy [26]. As for the dose of LIPUS as treatment for tissue repair, Fung CH et al proved that LIPUS with intensity of 30 mW/cm^2 could promote bone volume and woven bone ratio in comparison with control group, while LIPUS with intensity of 15 mW/cm^2 could not, indicating that the appropriate intensity selection was crucial in the therapeutic effect of LIPUS [27].

LIPUS increased secretion of PGE (2) and NO from MLO-Y4 cells at the time within 24 h following LIPUS stimulation, which might be the mechanism of LIPUS controlling proliferation and differentiation of osteoblasts [28]. In addition to accelerate the process of bone fracture, LIPUS also could significantly alleviate the periprosthetic osteolysis due to polyethylene in the distal part of the femur canal of rabbit model [29]. Ogawa T et al reported that LIPUS together with bone marrow transplantation was apt to increase new bone formation [30]. As for comparing the effect of bone repair, Oliveira P et al reported that both Ultrasound (US) and low-level laser therapy (LLLT) advanced the process of bone repair in rats, and there was no significant difference of accelerating bone healing ability between the two methods [31]. The ultrasound therapy promoted the expression level of osteocalcin at 7 and 13 days following upper third of the tibia excision surgery, indicating that osteocalcin was apt to involve in the bone healing promotion mechanism by ultrasound [32]. Katano M et al reported that LIPUS shortened the femur fracture healing period and endochondral ossification in the process of bone healing, involving in endothelial cell migration and neovascularization near fracture areas [33].

Based on the results of three-dimensional quantitative micro-CT and morphometric parameters, Tobita K et al reported that LIPUS decreased the time length for remodeling of callus and promote the mineralization of callus in rabbits [34]. According to the results of microfocus computerized tomography (micro-CT) and histologic examination, LIPUS promoted bone reossification of calvarial flat bone defects in rat compared to without LIPUS therapy [35]. Hui CF et al reported that there were osteochondral bridging and endochondral ossification between the cartilaginous and osseous tissues in LIPUS treatment group following spinal fusion surgery in rabbit, indicating that LIPUS was apt to accelerate spinal fusion by regulating osteointegration between the host bone and implanted materials [36]. Fávaro-Pípi E et al reported that LIPUS accelerated bone fracture recovery and promoted expression level of osteogenic genes in rats, including bone morphogenetic protein 4 (BMP4), osteocalcin and Runx2, especially at the late stage of bone repair [37]. Shakouri K et al reported that low-intensity pulsed ultrasound significantly increased callus mineral density with an insignificant promotion in the fractured bone strength [38].

LIPUS on osteoporotic fracture

Cheung WH et al reported that LIPUS promoted not only normal fracture healing but also osteoporotic fracture healing, and the osteoporotic treatment group showed better results in callus width, stiffness measurement and response of endochondral ossification than normal group in closed femoral fracture rats [39]. Cheung WH et al reported that LIPUS promoted osteoporotic fracture healing via activating callus formation, angiogenesis and callus remodeling, involving up-regulation of Col-1, bone morphogenetic protein-2, vascular endothelial growth factor and osteoprotegerin [40].

Ferreri SL et al reported that LIPUS improved load bearing characteristics, including trabecular mechanical strength and apparent level elastic modulus in estrogen deficient rat model of osteopenia, indicating that LIPUS might be an alternative therapy method for osteopenia [41]. In order to compare the effect of promoting bone healing in rabbits with distraction osteogenesis between LIPUS and low-level laser therapy (LLLT), Kocyigit ID et al

reported that LIPUS increased the mean BMD values at both 30 and 60 days postoperatively while LLLT just promoted the mean BMD values at 60 days postoperatively, demonstrating that both LIPUS and LLLT were helpful for distraction osteogenesis treatment [42]. As we knew, diabetes mellitus might delay the bone healing process and even lead to nonunion. Therefore, the intervention which could accelerate bone repair was required for the patients with diabetes mellitus following bone fracture. For the patients with type 1 diabetes mellitus (DM), LIPUS promoted the growth factor expression, the formation of cartilage and neovascularization in Wistar rats, indicating that LIPUS might exert beneficial effects on bone fracture patients with risk factors which might delay bone healing [43].

LIPUS on osteoarthritis

Gurkan I et al reported that PLIUS alleviated surface irregularities, decreased matrix loss degree with the inhibition of TGF-beta1 production, and attenuated the proceeding of cartilage degeneration in Hartley guinea pigs, which might be an alternative therapy method for human osteoarthritis [44].

LIPUS down-regulated the Mankin scores and the expression level of MMP-13 in rabbit articular cartilage of animal model of Osteoarthritis [45] Low-intensity pulsed ultrasound (LIPUS) decreased MMP13 mRNA expression in cultured chondrocytes and down-regulated the mRNA expression of MMP13 and MMP1 in articular cartilage explants, indicating that LIPUS could inhibit inflammation via regulating MMP mRNA expression so as to protect articular cartilage [46]. LIPUS together with dimethylsulfoxide gel could reduce JNK phosphorylation and protein expression levels of TNFα, IL-1β, NFκB, inhibiting inflammatory response following traumatic muscle injury [47].

As for the role of cyclooxygenase-2 (COX-2) in the process of LIPUS treatment on endochondral bone healing, Naruse K et al reported that LIPUS significantly shortened the endochondral bone remodeling period for wild-type mice, but LIPUS could not exert such effect on cyclooxygenase-2 knockout mice, demonstrating that COX-2 played a key role in the process of LIPUS treatment on endochondral bone healing [48]. The hyaluronan (HA) played an important beneficial role for normal synovial joint function. LIPUS intervention promoted the level of HA synthase (HAS) 2,3 which activated HA secretion and reduced the level of hyaluronidase (HYAL) 2 which inhibited HA secretion, resulting in cumulation of high-molecular weight HA, indicating that LIPUS might be an effective therapy method for inflammatory joint diseases, for instance osteoarthritis [49].

Li X et al reported that LIPUS therapy accelerated cartilage repair in rabbit knee osteoarthritis via reducing the expression level of p38, ERK1/2 and MMP-13 [50]. LIPUS could obviously depress the proliferation of HIG-82 which was stimulated by proinflammatory cytokines. Moreover, LIPUS decreased histological lesions in knee joints of MRL/lpr mice. Therefore, LIPUS tended to be a possible treatment for inflammatory knee joint diseases [51].

In conclusion, LIPUS is used widely in orthopedic practice, including bone and soft tissue healing process. More clinical trials are required to confirm the effect of LIPUS on human being. If the therapeutic effect is proved to be true and reliable, many patients with orthopedic diseases will achieve benefits from LIPUS.

Disclosure of conflict of interest

None.

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