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● *Original Contribution*

EFFECT OF LOW-INTENSITY PULSED ULTRASOUND ON JOINT INJURY AND POST-TRAUMATIC OSTEOARTHRITIS: AN ANIMAL STUDY

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Abstract—This study investigated the therapeutic potential of low-intensity pulsed ultrasound (LIPUS) in post-traumatic osteoarthritis (PTOA). Intra-articular fracture of the medial tibial plateau was surgically created in 30 rats. LIPUS was applied to the operated joints either for the first 2 wk ($LIPUS_{1-2}$ group) or in weeks 4 and 5 after intra-articular fracture ($LIPUS_{4-5}$ group). In controls, the operated knees were not treated with LIPUS ($LIPUS_0$ group). The rats were monitored with weekly gait analysis and euthanized at week 8. Among the altered gait parameters, the maximal and average paw print areas in the $LIPUS_{1-2}$ and $LIPUS_{4-5}$ groups, but not the $LIPUS_0$ group, had either reached baseline or significantly recovered (70%, $p < 0.05$) by week 8. PTOA pathology in both the $LIPUS_{1-2}$ and $LIPUS_{4-5}$ groups was less severe than that in the $LIPUS_0$ group (Mankin score: 5.4 and 4.5 vs. 8.8, $p < 0.05$). In conclusion, LIPUS treatment partially improved the gait of the affected limbs and reduced cartilage degeneration in PTOA. (E-mail:)

Key Words: **Post-traumatic osteoarthritis, Ultrasound, Cartilage, Gait, Intra-articular fracture.**

INTRODUCTION

Post-traumatic osteoarthritis (PTOA) accounts for about 12% of symptomatic osteoarthritis (OA) cases and is difficult to manage clinically (Brown et al. 2006; Buckwalter and Brown 2004; McKinley et al. 2010). Joint injury disrupts tissue matrix, causes cell death and bleeding, and triggers traumatic inflammation (Lotz and Kraus 2010). Inflammatory cytokines are detrimental to the integrity of articular cartilage as they activate matrix-degradation enzymes, inhibit matrix production and induce chondrocyte apoptosis (Lotz 2001). In severely injured joints, increased inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), in joint fluid could persist for as long as 6–12 mo and deliver secondary damage to the joint—the onset of PTOA (Marks and Donaldson 2005).

In the tissue, low-intensity pulsed ultrasound (LIPUS) produces no thermal effects, but generates mechanical stresses that stimulate a range of cellular responses to promote cell proliferation and tissue formation (Zhang et al. 2003a). In cartilage, LIPUS stimulates chondrocyte proliferation and matrix production in various experimental settings (Choi et al. 2006; Ikeda et al. 2006; Korstjens et al. 2008; Naito et al. 2010; Zhang et al. 2002, 2003b). LIPUS does not induce chondrocytes in hyaline cartilage to terminal differentiation (Zhang et al. 2002). This is important because terminally differentiated chondrocytes eventually become apoptotic, a common pathology of OA (Hwang and Kim 2015).

LIPUS suppresses inflammation in several ways: reduction of leukocyte infiltration; augmentation of macrophage phagocytosis; and suppression of TNF-α and IL-1β expression by synovial cells (Nakamura et al. 2011; Signori et al. 2011; Zhou et al. 2008). In addition, LIPUS treatment inhibited the activity of matrix metalloproteinase 13 (MMP-13) and improved OA pathology in animal models (Gurkan et al. 2010; Li et al. 2011; Zeng et al. 2012).

To examine the effect of LIPUS on PTOA, a valid animal model of joint injury is essential. Surgical meniscectomy and/or transaction of the anterior cruciate ligament to destabilize the knee and shift focal loading stresses on articular cartilage are the most common approaches to induction of PTOA in animals (Li et al. 2011;

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Zeng et al. 2012). Intra-articular fracture, where both bone and articular cartilage are fractured, is a type of severe joint injury. Concomitant intra-articular fracture increases the risk of PTOA of an injured joint as much as 20-fold (Schenker et al. 2014). Intra-articular fracture, including both closed (Furman et al. 2007) and surgical (Llinas et al. 1993; Trumble and Verheyden 2004), has been used in animal studies to induce PTOA. An open surgery ensures the accuracy and consistency of intra-articular fracture produced in the joints, and the simultaneously applied fracture fixation simulates clinical management of the intra-articular fracture (Zahoor et al. 2016). The outcome and pathology of a PTOA model are often evaluated with end-of-the-study histology, although joint pathology evolves from injury to degeneration. Gait reflects joint injury and cartilage degeneration in the lower extremity with alterations of a group of gait parameters (Naili et al. 2017). In follow-up of the rats with knee OA, gait analysis sensitively and accurately detected antalgic and shuffling gait in accordance with the stages of the OA development (Jacobs et al. 2017). Gait analysis is particularly valuable for animal models of PTOA, where clinical symptoms and physical signs are lacking.

Although LIPUS enhances fracture healing and improves cartilage repair (Bayat et al. 2017; Cook et al. 2001; Heckman et al. 1994), whether LIPUS treatment prevents or delays the development of PTOA secondary to intra-articular fracture is unknown. In this study, intra-articular fracture was surgically created in rat knees for PTOA induction. LIPUS was applied to the rat knees with intra-articular fracture at either the early stage of injury or the later osteoarthritic stage. The effects of LIPUS on PTOA development were longitudinally monitored with gait analysis and evaluated with histology at the end of the study.

METHODS

In this study, 30 male Sprague-Dawley rats at 12 wk of age were used (approved by MedStar Health Research Institute Institutional Animal Care and Usage Committee). The Sonic Accelerated Fracture Healing System (Bioventus, Durham, NC, USA) delivered ultrasound at an intensity of 30 mW/cm^2 (spatial-average, temporal-average intensity), with a sinusoidal waveform of frequency 1.5 MHz. The pulse burst frequency was 1 kHz, with a burst duration of 200 μs. The devices were calibrated by the manufacturer before applications and validated at the completion of this study.

Intra-articular fracture model

The rats were anesthetized by inhalation of isoflurane. After skin preparation, a para-patellar incision was made on the left knee joint of a rat to expose the medial tibial plateau as described previously (Zahoor et al. 2016). The middle point between the tibial anterior crest and the outermost edge of the medial plateau was used as a reference of osteotomy. A surgical blade (#11) was placed at the midpoint of the medial tibial plateau and in perpendicular to the front edge of the joint surface. The medial tibial plateau was osteotomized vertically, including the covering articular cartilage. The fractured medial plateau was reduced and fixed with two needles (23 gauge) transversely. After the wound was closed, rats were returned to their cages without immobilization. All animals survived the surgical procedure and exhibited no signs of infection.

Application of LIPUS

Under anesthesia, the rats were shaved around the operated left knees. Coupling gel was applied on the skin on the medial side of the knees, where an ultrasound transducer was secured in place with tape (Fig. 1). For the experimental groups, the LIPUS device was activated for 20 min/d, 5 d/wk, for 2 consecutive wk (Wang et al. 1994). For the control group, the device was affixed to the rat knees as scheduled for the experimental groups, but was not activated.

For LIPUS treatment, 30 rats with an intra-articular fracture of the medial tibial plateau were divided into three groups evenly. In the LIPUS$_{1\text{-}2}$ group, LIPUS was applied to the operated knees for the first 2 wk, starting on the second day post-surgery. In the LIPUS$_{4\text{-}5}$ group, LIPUS was applied for 2 wk, during weeks 4 and 5 after creation of the intra-articular fracture. In the LIPUS$_0$ group,

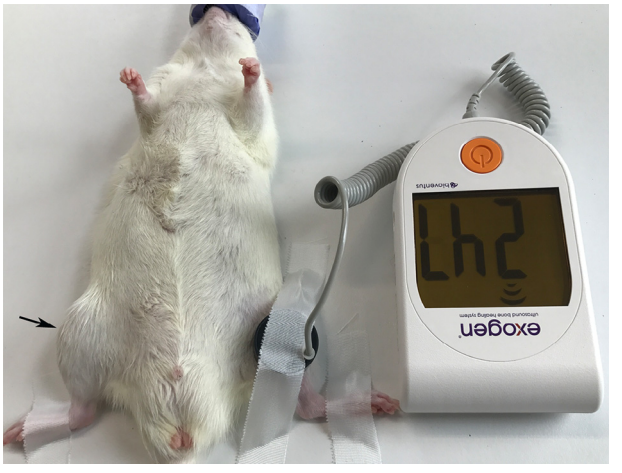

Fig. 1. Setup for application of LIPUS. Under anesthesia, the rat is in supine position and the hind limbs are secured to the table with hip in full abduction and knee in 90° bending. The transducer of the ultrasound device is attached to the medial side of the knee with a piece of tape. The transducer is positioned directly above the intra-articular fracture on the medial tibial plateau. The *arrow* indicates the level of the opposite knee.