Ultrasound in Med. & Biol., Vol. ■■, No. ■■, pp. ■■–■■, 2018

0301-5629/$ - see front matter

https://doi.org/10.1016/j.ultrasmedbio.2018.02.002

● *Original Contribution*

EFFECTS OF LOW-INTENSITY PULSED ULTRASOUND FOR PREVENTING JOINT STIFFNESS IN IMMOBILIZED KNEE MODEL IN RATS

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(*Received* 4 *October* 2017; *revised* 30 *January* 2018; *in final form* 3 *February* 2018)

Abstract—The purpose of this study was to examine the effect of low-intensity pulsed ultrasound (LIPUS) in preventing joint stiffness. Unilateral knee joints were immobilized in two groups of rats (n = 6/period/group). Under general anesthesia, the immobilized knee joints were exposed to LIPUS for 20 min/d, 5 d/wk, using an existing LIPUS device (LIPUS group, 1.5-MHz frequency, 1.0-kHz repetition cycle, 200-μs burst width and 30-mW/cm^2 power output) until endpoints (2, 4 or 6 wk). In the control group, general anesthesia alone was administered in the same manner as in the other group. The variables compared between the groups included joint angles; histologic, histomorphometric and immunohistochemical analyses; quantitative reverse transcription polymerase chain reactions; and tissue elasticity. LIPUS had a preventive effect on joint stiffness, resulting in decreased adhesion, fibrosis and inflammation and hypoxic response after joint immobilization. (E-mail: hagi@med.tohoku.ac.jp)

Key Words: **Joint immobilization, Low-intensity pulsed ultrasound, LIPUS, Joint stiffness, Joint contracture, Adhesion, Inflammation, Hypoxia, Fibrosis, Elasticity.**

INTRODUCTION

Joint immobilization is a common treatment option for musculoskeletal disorders such as fractures, sprains, arthritis and tendonitis (Duthie 1952; Gault and Spyker 1969; Partridge and Duthie 1963; Swanson 1956). It is frequently used to limit local dynamic motion and reduce joint pain, swelling and inflammation (Salter and Field 1960; Trudel et al. 2000).However, long-term joint immobilization causes undesirable side effects such as joint stiffness (Akeson et al. 1987). Joint stiffness is defined as a decrease in both active and passive ranges of motion (ROMs) of the joint (Trudel et al. 2000). Once the joint becomes stiff, it is extremely difficult to recover full ROM even after sufficient rehabilitation, surgical intervention or other interventions (Trudel et al. 2001). The pathology of joint stiffness has been studied, and its causes are divided mainly into two categories: those of the muscle (myogenic) and those of the periarticular structures (arthrogenic) (Enneking and Horowitz 1972; Evans et al. 1960; Trudel and Uhthoff 2000). Recent studies have revealed that the arthrogenic factors, especially those within the joint capsule, play an important role in the progression of joint stiffness after immobilization (Hagiwara et al. 2012; Hildebrand et al. 2004; Kanno et al. 2010; Moriyama et al. 2006; Trudel and Uhthoff 2000). After a release of the joint capsule in joint stiffness, ROM was noted to return to almost the normal range, which indicates that the joint capsule was one of the most important factors in joint stiffness (Chimoto et al. 2007; Hagiwara et al. 2006). Although the changes in the joint capsule after immobilization have not been clarified yet, adhesion, fibrosis, inflammation and hypoxic conditions have been proposed as possible pathologies (Bunker and Anthony 1995; Matsumoto et al. 2002; Mattyasovszky et al. 2010; Rodeo et al. 1997).

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Conflict of Interest: The authors declare no conflicts of interest.

Low-intensity pulsed ultrasound (LIPUS) is a type of ultrasound that emits pulsed waves at lower intensities (<3 W/cm^2), and its effect on fractures was first reported by Duarte (1983). Subsequently, many studies were conducted to investigate its effects, and it is generally accepted as a tolerable option in the treatment of bone fractures (Warden et al. 2000). Additionally, other effects of LIPUS on various cell types, such as cementoblasts (Dalla-Bona et al. 2006, 2008), periodontal ligament cells (Harle et al. 2001; Inubushi et al. 2008), synovial membrane cells (Nakamura et al. 2010) and chondrocytes (Takeuchi et al. 2008), have also been reported. Furthermore, LIPUS has beneficial effects such as reduction of inflammatory responses (Nakamura et al. 2011), restoration of angiogenic cells, induction of angiogenesis and improvement in hypoxic conditions (Hanawa et al. 2014). Nakamura et al. (2011) reported that LIPUS suppressed the proliferation and growth of rabbit synovial fibroblasts stimulated with interleukin-1β (IL-1β) or tumor necrosis factor-α (TNF-α), reduced cyclooxygenase-2 (Cox-2) expression and decreased the overall inflammatory response. Hanawa et al. (2014) reported that LIPUS upregulated the expression of vascular endothelial growth factor (VEGF), endothelial nitric oxide synthase (eNOS) and basic fibroblast growth factor (bFGF) and increased capillary density and regional blood flow. From these studies, we hypothesized that LIPUS would reduce inflammation and improve hypoxic conditions in the joint capsule after immobilization, resulting in the prevention of joint stiffness.

Therefore, the aim of this study was to explore the potential of LIPUS in preventing joint stiffness in immobilized knees in rats.

METHODS

Experimental design and surgical procedure

Animals. Ethical approval of this study was obtained from the Animal Research Committee of Tohoku University (Approval No. 2013 MdA-360).

Male mature Sprague-Dawley rats, aged 12 wk, were used in this study (CLEA Japan, Tokyo, Japan) to avoid estrous cyclicity as reported previously (Chimoto et al. 2007; Hagiwara et al. 2006, 2008; Trudel et al. 2000). General anesthesia was induced by intraperitoneal injection of sodium pentobarbital (50 mg/kg), and the right knee joints were immobilized with an internal, but extra-articular, fixator at 150° of flexion according to previous reports (Hagiwara et al. 2006, 2010). A rigid plastic plate implanted subcutaneously fixed the proximal femur and the distal tibia away from the knee joint and was held in place with one metal screw at each end (Fig. 1). The knee joint capsule and the joint itself were untouched. The rats were randomly divided into two groups, the LIPUS and control groups. Under inhalational general anesthesia with isoflurane, the LIPUS group received LIPUS on the immobilized knee joint from the lateral side for 20 min/d, 5 d/wk, until the endpoints of the study periods (2, 4 and 6 wk). LIPUS was applied using a commercially available device (SAFHS Teijin, Tokyo, Japan; frequency = 1.5 MHz, repetition cycle = 1.0 kHz, burst width = 200 μs and power output = 30 mW/cm^2). LIPUS was focused just around the knee joint, and there was no interference with the internal fixator (Fig. 1). The control group received general anesthesia alone without LIPUS in the same manner as the LIPUS group. Thirty-six rats were prepared for histologic analyses, 36 for joint angle measurement and 36 for quantitative reverse transcription polymerase chain reaction (qRT-PCR) (n = 6/each period/each group). This model had restricted ROM, primarily in extension, as well as prominent capsular changes in the posterior side of the joint capsule (Chimoto et al. 2007; Hagiwara et al. 2006, 2008; Trudel et al. 2000). Each sample was assessed in a blinded manner.

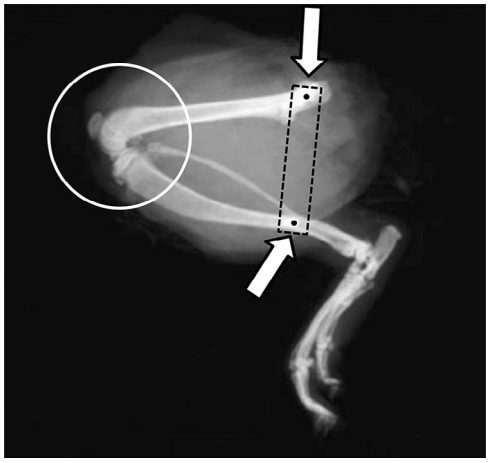

Fig. 1. X-Ray image of a knee joint after immobilization. The knee joints were immobilized with an internal, but extra-articular, fixator at 150° of flexion. A rigid plastic plate implanted subcutaneously fixed the proximal femur and distal tibia away from the knee joint and was solidly held in place with one metal screw at each end. The knee joint capsule and the joint itself were untouched. *Arrows* indicate the positions of the metal screws. The *broken line* indicates the position of the plastic plate. The *circle* indicates the position of the probe of low-intensity pulsed ultrasound.

Joint angle measurements. Joint angles were measured with a custom-made device after taking lateral X-rays of the knees according to previous reports (Ando et al. 2012; Chimoto et al. 2007; Onoda et al. 2014). After euthanasia by intraperitoneal injection of an overdose of sodium pentobarbital, the right limb was divided at the hip joint and the muscles were removed while preserving the peri-articular tissues around the knees (Ando et al. 2012; Chimoto et al. 2007; Onoda et al. 2014). Three different torques (450, 900 and 1350 g·cm) were chosen to extend