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Effect of high frequency electromagnetic wave stimulation on muscle injury in a rat model

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ABSTRACT

Introduction: The aim of this study was to investigate biological changes in tissues with muscle contusion after the application of high frequency (HF) electromagnetic wave.
Methods: An acrylic pipe was placed on the right hind limb and a metallic ball was dropped inside the pipe, which resulted in a muscle contusion. After acquiring the optimal condition for muscle contusion, 20 Sprague-Dawley rats were allocated to the HF treatment (N = 10) and sham groups (N = 10), which then underwent muscle contusion injury at their right thigh. The thickness and circumference of the right thigh and the left thigh (negative control groups) were measured (day 0). HF electromagnetic wave stimulation for three days was performed on the contusion area in the HF group after one day. Thickness was measured at the thickest area of both hind limbs and the circumference was measured every day for three days. The sham group received no treatment, and the circumference and thickness were measured using the same method. After three days, Hematoxylin and eosin and immunohistochemical (IHC) staining for IL-1β were performed and TUNEL assay was conducted for apoptosis in the skin and muscle layers.
Results: The thigh muscle thickness at day 1 was significantly different between groups ($P = 0.018$) and this difference was observed between both sham and control groups (corrected $P = 0.007$), and between sham and HF groups (corrected $P = 0.043$). Thigh circumference was significantly different at day 3 ($P = 0.047$) and this difference was found between sham and control groups (corrected $P = 0.018$), and between sham and HF groups (corrected $P = 0.032$). In the HF group, the inflammatory response was reduced to almost the same level as the control group. Evaluation of IL-1β level, the inflammatory cytokine, through IHC showed marked localization of IL-1β in muscle fibers of the sham group. However, significantly less IL-1β was observed in the muscle of the HF treatment group. There was neither injury nor apoptosis after HF stimulation.
Conclusions: Application of the HF showed therapeutic effect on muscle contusion by reducing muscle swelling. This effect might be caused by the anti-inflammatory action of the HF, which evoked energy into the injured muscle.

Introduction

High frequency (HF) electromagnetic waves reside between 3 and 30 MHz in the International Telecommunications Union frequency bands. HF electromagnetic waves are used in the cosmetics industry for skin collagen remodeling [1], herniated lumbar disc treatment [2], osteoarthritic knee pain [3], and intractable low back pain [4]. This radiofrequency wave transmits heat into the tissue to a variable degree according to the electrical properties, thermal properties, and blood flow [5].

Variable kinds of heat therapy including hot pack, infrared, ultrasound, and shortwave are used in the management of musculoskeletal pain. Many studies on the transmission of heat to tissues have been performed and the biological effects of heat established [5–7]. Heat therapy controls pain by reducing nociceptive activity [8], increasing blood flow [9] and muscle relaxation [10], and modulating inflammatory cytokines [11]. Heat

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therapy also enhances the absorption of muscle and subcutaneous contusions by increasing blood flow [12,13].

Although HF electromagnetic wave treatment has been used in clinical trials [4,14,15], its biological effect on tissues is not well understood. To apply HF electromagnetic wave treatment for musculoskeletal pain and injury, its thermal properties and biological effects must be thoroughly investigated. The changes in tissue after HF application, as well as the effect of HF electromagnetic wave on muscle contusion must be elucidated prior to clinical use. However, there has been no study on the effects of HF on muscle contusion, or the biological changes in tissues after HF electromagnetic wave application.

The aim of this study was to investigate biological changes in tissues with muscle contusion after the application of HF electromagnetic wave.

Materials and methods

Preparation of muscle contusion rat model

For the muscle contusion rat model, 15 male Sprague-Dawley rats weighing 300–370 g aged ten weeks (Orient Bio, Gapyeong, Korea) were used. After a one-week acclimation period, the rats were anesthetized with 2% isoflurane (Ifran®, Hana Pharm, Hwaseong, Korea). The anterior portions of the both hind limbs were shaved with an electric clipper. Rats were placed on the right side with fixation of the hind limb in a 90° flexed position. An acrylic pipe was placed on the right hind limb and a metallic ball was dropped inside the pipe, which resulted in a muscle contusion (Fig. 1). Rats were divided into three groups and five rats in each group underwent the experiment under each condition. Three conditions (200 g metallic ball with 30 cm drop height, 300 g metallic ball with 30 cm drop height, and 500 g metallic ball with 20 cm drop height) were tried to determine the conditions required for maximal contusion. Maximal contusion was determined when the most prominent swelling and bruise were found 5 min after single drop of the metallic ball.

Among the three injury conditions tested, the 300 g metallic ball and 30 cm drop height evoked the most prominent swelling of the thigh muscle (Fig. 2). Using the 300 g weight and 30 cm drop height, thigh circumference increased 21.7% at day 7 and 20.8% at day 14. Using the 200 g weight and 30 cm drop height, thigh circumference increased 1.3% at day 7 and decreased 3.8% at day 14. Finally, using a 500 g weight and 20 cm drop height, thigh circumference increased 2.5% at day 7 and decreased 4.9% at day 14. According to this result, we determined 300 g as the optimal ball weight and 30 cm as the optimal pipe height for the HF experiment.

High frequency wave treatment

After acquiring the optimal condition for muscle contusion, another new 20 rats were allocated to the HF treatment (N = 10) and control groups (N = 10), which then underwent muscle contusion injury at their right thigh after anesthetization with the above-mentioned method. After muscle contusion injury, the thickness and circumference of the right thigh and the left thigh (negative control) were measured (day 0). After the measurement, rats were left to move freely after waking from anesthesia. One day after the preparation of the muscle contusion model, electric conduction gel was applied and HF wave stimulation was performed on the contusion area in the HF group after anesthetization (Fig. 3A). HF stimulation used a frequency of 4.4 MHz, an intensity of 260 mA (35 W), with a 2 s on/2 s off pulse mode for 20 min every day for three days using a HF electromagnetic wave stimulator (HIPER-500, JS-on Co. LTd., Seoul, Korea). After stimulation, the conduction gel was removed and the circumference and thickness of both hind limbs were measured. Thickness was measured at the thickest area of the hind limb and the circumference was measured at three points. We determined just below the patella as the second point of the measurement, followed by 5 mm above and below the second point as the first and third points (Fig. 3B). Circumference measurements at the three points were averaged to determine the change in swelling states. The stimulation and measurement were repeated for three days with anesthetization during the experiment. The stimulation and measurement for one day took about one hour.

Fig. 1. For the muscle contusion rat model, he anterior portions of the both hind limbs were shaved with an electric clipper. Rats were placed on the right side with fixation of the hind limb in a 90° flexed position. An acrylic pipe was placed on the right hind limb and a metallic ball was dropped inside the pipe.

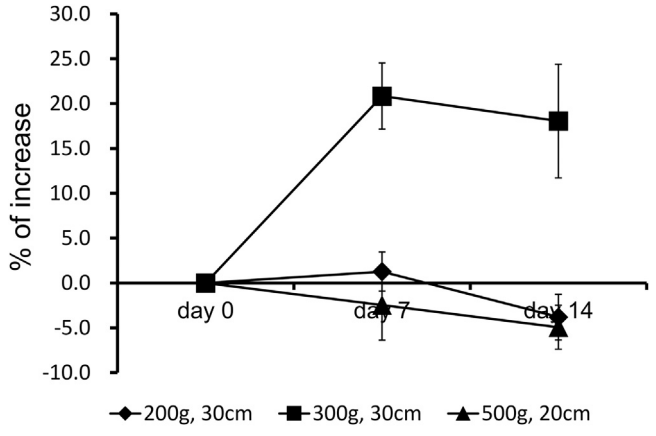

Fig. 2. Several drop heights and weights of the metallic ball were tried to determine the conditions required for maximal contusion. Among the three injury conditions tested, the 300 g metallic ball and 30 cm drop height evoked the most prominent swelling of the thigh muscle (% of increase in thigh circumference).

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