Introduction: Strain injuries are the most common injuries in sports that require sprinting or jumping. These injuries are often located near the muscle tendon junction. As with most traumatic muscle injuries, recovery from muscle strain involves a lengthy rehabilitation period with increased risk for reinjury (1,2). Even though muscle tissue retains its ability to regenerate after injury, the healing process involved has been found to be slow and often incomplete. Low-Intensity Pulsed Ultrasound (LIPUS) is in clinical use for the enhancement of fracture healing. Animal models demonstrate LIPUS could improve tendon healing by facilitating fibroplasia and protein synthesis (3,4). However, there is no any report about LIPUS effects on muscle healing. The purpose of this study was to use in vitro and in vivo analyses to assess the effect of LIPUS on the muscle healing after strain injury in a mice model.

Materials and Methods: In vitro: The C2C12 cells, a line derived from mouse muscle myoblasts were cultured. All cells were exposed to LIPUS therapy using EXOGEN 2000+ system Ultrasound apparatus (Piscataway, NJ) with a total treatment of 20 minutes per 48 hours. At intervals of 2, 4, 6, and 8 days cell growth was measured by increase in cell number and Western Blot analysis of myogenin and actin (myogenesis marker).

In Vivo: Forty mice (C57BL10J+/+) were divided into five groups of eight animals each, using in our published strain injury model (5). The control group did not undergo LIPUS therapy. The US 7 days group was treated with LIPUS for continuous 7 days after laceration; US 14 days group for continuous 14 days; US 21 days group for continuous 21 days; and US 28 days group for continuous 28 days of LIPUS therapy (20 minutes/each day). All animals were sacrificed at 4 weeks after the strain injury. Evaluation methods include: muscle regeneration and muscle contractile properties. A Student’s t-test was used for statistical analysis. Statistical significance was defined as P<0.05.

Results: In vitro: The cell growth curve showed that the experimental group treated by LIPUS had significantly proliferative rate and cell number at day 6 and 8 (p<0.05) (Fig 1). Densitometric evaluation showed increase in myogenin and actin proteins in cells treated with LIPUS in day 4, 6, and 8 groups (Fig 2).

In Vivo: The study demonstrated an increased number of regenerating myofibers in all the LIPUS-treated groups when compared with the controls. However, only US 21 and 28 days group led to a significantly higher number of regenerating myofibers when compared to the control animals (P<0.05) (Fig 4). Physiologic Evaluation of Muscle Contractile Properties after LIPUS therapy: There was a significant difference between the control group and the US 21 and 28 days group for the results of fast twitch and tetanus muscle strength (Fig 5 and 6).

Discussion: Strain injuries are especially common in sports that require sprinting or jumping. These injuries are often located near the muscle tendon junction. As with most traumatic muscle injuries, recovery from muscle strain involves a lengthy rehabilitation period with increased risk for reinjury. Ultrasound has many medical applications, including therapeutic, operative, and diagnostic procedures. Ultrasound is used both operatively and therapeutically, with intensities ranging from 0.2 to 100 W/cm. In this study, we select the LIPUS apparatus: EXOGEN 2000+ system Ultrasound apparatus (Piscataway, NJ), is a FDA proof for clinical use. The ultrasound quality and stability are very stable and the machine is easy to operate and have own battery system.

The results of this study suggest that LIPUS therapy enhances regenerative myofiber formation in injured muscles. The repaired muscles had better physiologic performance and earlier histological evidence of healing in the LIPUS -treated group. However, the effective result is depended on intensity and dosage of LIPUS. LIPUS therapy may have great clinical potential for use in shortening the healing time in injured muscle.


Acknowledgements: Acknowledgments

The authors are grateful to National Science Council of the Republic of China for financially supporting this study under Contract No. NMRPG340101 NSC94-2314-B-182A-049.