THE EFFECT OF RELAXIN ON MUSCLE RECOVERY AFTER STRAIN INJURY

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Introduction
Skeletal muscle injuries are common problems in traumatology and orthopaedic surgery. Although injured muscle can regenerate, the healing process is very slow and incomplete. The development of scar tissue appears to preclude the complete recovery of muscle after injury [1]. We have shown that the administration of relaxin, a polypeptide cytokine/growth factor, can improve muscle regeneration and inhibit fibrous tissue formation after laceration injury [2]. However, muscle strains are the most common muscle injuries encountered in orthopaedic surgery. Therefore, in this study we developed a strain injury model and used it to investigate the effect of relaxin on muscle recovery after strain injury.

Methods
Twenty-four 8-week-old mice (C57BL/6J) were used for this study. A muscle strain injury model based on the model described by Garrett [3] was developed in mice. A small incision was used to expose the distal tendon of each tibialis anterior (TA) muscle at its insertion point on the foot. 6-0 nylon suture was passed through the tendon and attached to a force gauge, and the entire muscle tendon was pulled to 300g at the rate of 1 cm/minute. These mice then were divided into 3 groups based on time of relaxin or PBS (control) injection (1 week, 10 days, or 2 weeks). In each group, the left and right TA muscles were injected with either 100ng of relaxin in 5μl PBS or 5μl PBS alone (control group). All mice were sacrificed 4 weeks after strain injury, and all TA muscles were harvested for histological assessment. An ANOVA test was used for statistical analysis.

Results
We observed more regenerating myofibers at the strain injury site of the relaxin-injected muscles than at the strain injury site of the PBS-injected (control) muscles at all time points (Fig. 1). Indeed, the injection of relaxin 10 days or 2 weeks after strain injury led to the regeneration of significantly more myofibers than did injection of PBS at these time points (p<0.05; Fig. 1). However, our comparison of the relaxin-injected muscles revealed no outcome differences based on time of relaxin injection. Trichrome staining revealed that relaxin-injected muscles contained significantly less fibrous scar tissue than did control muscles at all time points (p<0.05; Fig. 2).

Discussion
The injection of relaxin increased the number of regenerating myofibers and decreased the area of fibrous scar tissue at the strain injury site, so the histological results from this study suggest that the injection of relaxin can improve muscle healing after strain injury.

Injured muscle undergoes the following phases of healing: degeneration and inflammation, regeneration, and fibrosis. Muscle regeneration begins to take precedence during the first week after injury and peaks at about 2 weeks after injury [4]. Fibrosis generally begins during the second week after muscle injury and increases over time [4]. TGF-β is considered a key factor in the development of fibrosis in various tissues [5] and acts during inflammation and fibrosis to stimulate the production of extracellular matrix proteins and to inhibit their degradation [6]. Relaxin attenuates the actions of profibrotic cytokines, including TGF-β and interleukin-18 [7]. The current study revealed that the injection of relaxin 10 days or 2 weeks after injury significantly increased the number of regenerating myofibers in injured muscle. Furthermore, the injection of relaxin 1 week, 10 days, or 2 weeks after injury significantly decreased the amount of fibrous scar tissue that formed in the injured muscle. This result reflected that relaxin promoted regeneration myofibers at 10days and 2weeks and probably inhibited TGF-β and prevented fibrosis. To further evaluate the function of relaxin in injured muscle, our future investigation will include longer-term analysis.

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References

Fig.1 The number of regenerating myofibers (*p<0.05)

Fig.2 Area of fibrous scar tissue (*p<0.05)