INHIBITION OF PERIPHERAL NERVOUS SYSTEM ALTERS LIGAMENT HEALING

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INTRODUCTION

Ligament injuries occur frequently in all age groups at all activity levels [1]. The healing potential varies widely among ligaments, but even in ligaments that heal well, there are mechanical, biochemical, and morphological alterations which may exist for a number of years post-injury [2]. Cell migration and proliferation are critical responses for wound healing, and there is mounting evidence that peripheral nervous system agents, neuropeptides and neurotransmitters, play an essential role in orchestrating these responses [3, 4]. In particular, the sensory neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP) have been shown to play an important role in healing of wounds in bone, skin, and cornea [5, 6]. Cell culture studies also indicate that these neuropeptides increase cellular proliferation [4]. In the MCL, sensory nerves are often associated with blood vessels and can influence ligament blood flow [7]. Following gap injury, the distribution and immunoreactivity of SP and CGRP is also changed [7]. Recent research also implicates denervation with the onset of osteoarthritis and nerve injury to affect ligament healing [8, 9]. Furthermore, since these neuropeptides are associated with pain and inflammation at the site of injury, inhibitors of sensory innervation are used to treat pain. One of these inhibitors, capsaicin, is used for the treatment of orthopedic disorders, including rheumatoid arthritis and osteoarthritis [10]. Although strong evidence exists that the sensory nervous system influences soft tissue healing, no studies, to our knowledge, have shown if sensory inhibition affects ligament healing. The purpose of this study was to investigate the role of the sensory nerves in ligament healing using an inhibitor of sensory innervation in a rat MCL model.

METHODS

Capsaicin Treatment and Surgical Procedure

Six female Wistar rats (250g to 320g) were divided into two groups. All rats were anesthetized with isofluorane, and premedicated with subcutaneous injections of glycopyrrolate (0.02 mg/kg), butorphanol (0.5 mg/kg) and intraperitoneal injections of atropine (0.2 mg/kg) and terbutaline (0.2 mg/kg). To each group, either capsaicin (50 mg/kg, prepared by emulsification in 10% Tween 80 and 10% ethanol in sterile 0.9% saline; n=4) or sterile 0.9% saline (n=2) was administered by subcutaneous injection once per day for three consecutive days. On the last day of treatment, each rat underwent a complete surgical rupture of the MCL to one knee. The contra-lateral knee underwent sham surgery. Two weeks post knee surgery, rats were sacrificed using an overdose of pentobarbitone (150 mg/kg).

Mechanical Testing

Immediately following death, rats were stored at -80^{o}C until testing. Storage by freezing does not significantly alter biomechanical properties. On the day of testing, rat hind limbs were thawed at room temperature. The MCL of each leg was exposed and extraneous tissue was carefully dissected from the joint. MCLs, including intact femoral and tibial bone sections (FMT complex) were excised for *ex vivo* testing. Ligament area was then measured optically. The FMT complex was then mounted into a custom designed bath and optical markers were placed on the MCL near the insertion sites. Ligaments were preloaded (0.1 N) and preconditioned (1% strain for 10 cycles). Following preconditioning, ligaments were pulled to failure at a rate of 10% per second. Tissue force and displacement were recorded and ultimate stress and failure strain were calculated.

RESULTS

In the sham operated leg there were no significant differences in failure force, area, ultimate stress, or failure strain between capsaicin and control (saline) animals. Mechanical changes were noted in the ruptured side between treatment groups. One MCL failed to heal in the capsaicin treated group. In the remaining capsaicin treated animals, the failure force of the ruptured ligament was significantly lower ($p=0.037$; $n=3$) when compared to controls (Figure 1). Ultimate stress was also reduced, however this result was not significant ($p=0.091$; $n=3$). There were no changes in area or failure strain.

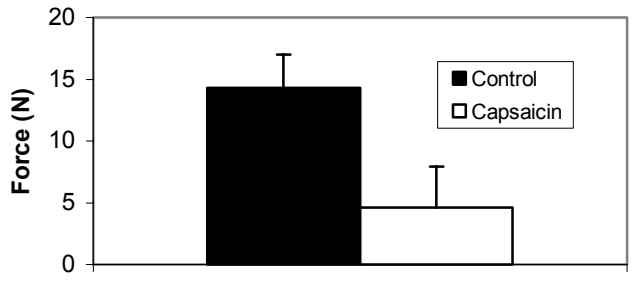

Figure 1: Following 2 weeks of healing, capsaicin animals had lower failure force in healing MCL's.

DISCUSSION

The results of this study provide compelling evidence that sensory innervation can influence ligament healing. Although capsaicin is an effective treatment of pain, this study establishes that it may have detrimental effects to healing soft tissues. Furthermore, since grafting of soft tissues is a common practice and grafting techniques focus primarily on damaged matrix, these result suggest that it is necessary to consider the regeneration of nervous tissue as well. Hence, understanding the role of innervation in ligaments is essential for improved understanding of ligament healing, the effect of denervation in grafting, and for developing increasingly sophisticated tissue engineering techniques.

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