Neuropeptide Regulation of Microcirculation in the Healing MCL Rabbit Model

INTRODUCTION:

Ligament and tendon injuries are a major source of morbidity, and can lead to persistent symptoms of pain, weakness or joint instability, with significant loss of quality of life. Currently, there is still no treatment that can speed healing, lessen morbidity or improve functional outcomes in ligament and tendon repair. Ligament healing occurs in 3 arbitrarily defined phases: Inflammation, followed by proliferation, and subsequent scar remodeling. It is now widely recognized that signaling molecules called neuropeptides are released from sensory and autonomic nerve endings, and play an important role in regulating the inflammatory response, the first phase of normal wound healing.

Substance P (SP), Calcitonin gene related peptide (CGRP), and Vasoactive Intestinal Peptide (VIP) are known neuropeptides that have been identified in joint tissues and are known to either promote or inhibit the inflammatory response in a variety of animal models of inflammation. Neuropeptides influence healing through regulation of blood flow, stimulation of macrophages and fibroblasts, and promotion of angiogenesis. Currently, no studies have looked at the influence of each neuropeptide on ligament healing in an in vivo setting. To understand each neuropeptide’s effect on ligament healing, an effective means of blocking the peptides action in vivo needs to be available. It is not known if inflammation is a necessary component of ligament healing. Since all current treatment recommendations for rest, ice, compression and elevation are targeted toward reducing inflammation, if a pro-inflammatory neuropeptide could be selectively blocked, without impairing the outcome of healing, the morbidity of ligament injury could be significantly reduced.

The purpose of this study was to determine the effect of selectively augmenting or inhibiting each neuropeptide on blood flow in the healing MCL at different times after injury, and to determine the optimal concentration of inhibitor that would block the action of each targeted neuropeptide on ligament blood flow.

METHODS:

8 adult female New Zealand rabbits were used. At Time 0, the MCL was surgically exposed, and the effects of topical application of neuropeptides and their selective antagonists on blood flow in the normal MCL were measured using Laser Speckle Perfusion Imaging (LSI). The data acquired at this time point was used as baseline control data for the uninjured MCL. In 6 of the rabbits, the MCL was transected in its mid substance, creating a 2mm gap. The 2 remaining rabbits were used as sham controls. At 2 wks, and at 6 wks, the same blood flow assays were performed. Change in perfusion for the injured MCL at 2wks and 6wks was compared to the uninjured baseline using Student’s t-test (n=6 per group; P < 0.05). The effects of agonist and antagonist (at concentrations 10^-8 mol and 10^-11 mol) on MCL blood flow were analyzed using Student’s t-test (n=6 per group; P < 0.05). All animals was treated and maintained in accordance with the guidelines of the Canadian Council on Animal Care.

RESULTS:

Mean perfusion of injured MCLs (n=6) at 2wks was 10.4+/-1.2 PU compared to 5.5+/-0.5 PU at baseline (p=0.01). Mean perfusion of injured MCLs (n=6) at 6 wks was 7.0+/-1.0 PU compared to 5.5+/-0.5 PU at baseline (p=0.06). SP antagonist 10^-8 mol effectively reduced SP vasoconstrictive activity by 3.6 +/- 3% (p=0.06). CGRP antagonist 10^-8 mol reduced CGRP 10^-7 mol vasoconstrictive activity by 6.6+/-1.3% (p=0.02) at 2 wks post injury. At 2wks VIP 10^-7 mol increased perfusion by 6.8+/-2%, compared to a perfusion change of -0.1+/-1% at 6 wks (p=0.06).

DISCUSSION:

This study examined the effects of selected neuropeptides on blood flow in the MCL injury model, using high resolution laser speckle perfusion imaging. LSI technology proved to be non-destructive, with rapid image acquisition, allowing repeated measures of blood flow at different times and under different conditions. Consistent with previous results, a marked increase in MCL blood flow was observed following injury. The changes in MCL perfusion with application of SP, CGRP, and VIP suggest that neuropeptides play a significant regulatory role in ligament blood flow.

SP displayed a vasoconstrictive effect on the medial collateral ligament at baseline. This response was diminished at two and six weeks following injury, possibly due to the immaturity of new vessels associated with angiogenesis, or perhaps is reflective of receptor saturation by increased amounts of endogenous SP released following injury, thus diminishing any response from the addition of exogenous SP. CGRP displayed a vasodilatory effect on the medial collateral ligament at baseline that was enhanced two weeks after injury, and diminished six weeks post injury. The vasodilatory role of CGRP both at baseline and two weeks following injury identifies CGRP as a key pro-inflammatory neuropeptide in the inflammatory phase of MCL healing.

This represents the first step into analyzing the influence of neuropeptides on ligament healing in vivo. Potential limitations of the study include the repeated surgical exposures required to obtain LSI perfusion maps, and the decision to arbitrarily choose a 100 fold increase in antagonist concentration as a means of blocking agonist action. Further dose response studies will be done to determine the optimal antagonist concentration required to block endogenous neuropeptide activity in vivo, in order to take the next step in this analysis, which would be to analyze the effects of continuous neuropeptide inhibition in an in vivo setting.

Nevertheless, this study has shown that neuropeptide effects on ligament blood flow can be manipulated, especially during the proliferative phase of healing. Additionally, CGRP has been identified to have vasodilator activity, suggesting it plays a more important role in inflammation than SP. Thus, CGRP may be a promising target for interventions to reduce the morbidity of ligament injury in the future.

ACKNOWLEDGEMENTS:

This work was supported by operating funds from the Canadian Institutes for Health Research. Dr. Bray is a Scientist of and Dr. Salo is a Senior Scholar of Alberta Innovates – Health Solutions.