THE EFFECT OF NKISK ON TENDON IN AN IN VIVO MODEL

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Introduction: The mechanism through which ligaments and tendons change length during growth and contracture is unclear. Investigation into a sliding fibril model for length change has shown that fibrils are discontinuous and overlap, are oriented in a specific direction and that length change occurs throughout the tendon. Additionally, in vitro studies into a putative "interfibrillar" bond that would allow fibril sliding in this model has shown that small polycations (gentamicin/polylysine) facilitate length change in vitro. Schmidt et al reported that NKISK, a polycationic pentapeptide with 2 lysines, corresponds to the binding site of decorin (a small proteoglycan that is found on the surface of collagen fibrils) for fibronectin, an adhesion molecule. In vitro studies in rat tail tendon have shown that NKISK potentiates creep similarly to gentamicin and polylysine. In this study, we hypothesize that NKISK will facilitate length change in an in vivo model.

Methods: Using an IACUC approved protocol, male Sprague-Dawley rats, 350-600 gms, were divided into three groups (n=10, 10 and 15 respectively). Under sterile conditions, the patellar tendon was percutaneously injected with NKISK in one ml of phosphate buffered saline (Group 1 = 1mM NKISK, Groups 2 and 3 = 5 mM NKISK). Under the same conditions, the contralateral/control patellar tendon was injected with one ml of PBS. The rats in Groups 2 and 3 were placed in a water tank for 2-5 minutes following injection to facilitate distribution of the injected fluid. Group 1 was sacrificed after 3, Group 2 after 4 and Group 3 after 7 daily injections. The patellar tendons, which have very distinct origins and insertions, were measured between these endpoints using a caliper accurate to 0.1 mm. The lengths of the experimental tendons were compared to the controls using a paired t-test and a percent change in length was calculated. One rat died in each group prior to completion of the injections and was not included in the statistical analysis.

After sacrifice, both hind limbs from Group 3 were amputated below the hip joint and frozen. The hind limbs were later thawed and the patellar tendons were isolated with the patellar and tibial attachments intact. Cross-sectional areas were assumed to be elliptical and estimated by measuring tendon width and thickness with calipers. The patellar tendons with bony attachments were loaded onto a servohydraulic materials testing machine and tensile loaded at 5 mm/min to failure. Using the force-deformation data, the maximum load, ultimate strength, slope, elastic modulus, displacement and strain at maximum load were calculated and the experimental tendons were compared to the control tendons using a paired t-test.

Results: NKISK resulted in a progressive increase in the length of the patellar tendons with both increase in concentration and treatment time. No significant change was noted in the tendons treated with 1mM NKISK. A statistically significant increase was noted in the lengths of the tendons treated with 5 mM NKISK for both 4 and 7 days (Groups 2 and 3), p = 0.002 and p < 0.001, respectively (see bar graph). The biomechanical analysis of the patella tendons in Group 3 showed no significant difference in the maximum load, ultimate strength, slope or elastic modulus. A statistically significant decrease was noted in the displacement to maximum load and strain at maximum load in the NKISK treated tendons, p = 0.007 and p = 0.01 (see table).

Discussion: The sliding fibril model for length change during growth and contracture in ligaments and tendons postulates manipulation of an “interfibrillar” bond to allow collagen fibrils to slide past one another. Previous studies have shown that small polycations can facilitate such sliding. In vitro studies with NKISK, a competitive inhibitor of the decorin/fibronectin binding site, in rat tail tendon have shown that it potentiates creep under tensile load. Decorin is thought to have a role in fibrillogenesis by being intimately associated with the fibrils. In our current study, which we believe to be the first to look at NKISK in an in vivo model, we demonstrate that NKISK significantly facilitates length change in rat patellar tendon and it appears that there is both a dose and length of treatment response. Furthermore, the biomechanical analysis demonstrated that NKISK changed the length of the tendon without weakening the material or structural properties of the tendon. However, the NKISK tendons (having already been strengthened) demonstrated decrease displacement and strain at maximum load. Although we are unable to look directly at the mechanism involved in the tendon lengthening in this experimental model, the implication is that NKISK is competing with decorin or other similarly structured molecules for interfibrillar binding sites in order to allow this length change. Clinically, this may eventually play a role in the treatment of tendon/ligament contracture. This study is a preliminary one, but we believe the findings are important enough to warrant further investigation.

References: