THE EFFECTS OF NKISK ON TENDON STRENGTH AND CREEP LENGTHENING

*Nixon, S; *Weinhold, P; +*Dahners, L
+*University of North Carolina, Chapel Hill, North Carolina. Department of Orthopaedic Surgery, The University of North Carolina, CB# 7055, Chapel Hill, NC 27599-7055, (919) 966-3340, Fax: (919) 966-6730, led@med.unc.edu

Introduction

The mechanism by which tendons and ligaments grow has been under investigation for some time. The sliding of discontinuous collagen fibrils past one another has been hypothesized as a mechanism for tendon and ligament growth (and contracture). The pentapeptide, NKISK, has been reported to induce creep length changes in rat tail tendons presumably by inhibiting the binding of fibronectin, an adhesion molecule, to decorin, a proteoglycan coding collagen fibrils (1). We hypothesize that decorin-fibronectin bonding is part of an “interfibrillar bond” and that competitive inhibition of this interfibrillar bond by NKISK facilitates the sliding of collagen fibrils (creep). As creep has been shown to cause a reduction in strength, it was our hypothesis that rat tail tendons treated with NKISK would be weaker than non-treated controls (2). Moreover, NKISK would induce higher rates of creep lengthening in treated tendons versus non-treated controls and, after creep, strength would be further decreased.

Methods

Tails harvested from 300-400g Sprague-Dawley rats sacrificed for unrelated IRB approved studies were frozen at -20°C. The rat tails were thawed for 30 minutes and then cut through a vertebral disc near the base of the tail and again 7 cm distally. Using micro forceps and a dissecting microscope, similar sized tendons were harvested from the rat tails. In each experiment, tendons from a single rat tail were used due to the fact that there is marked variability in the mechanical behavior of rat tail tendons between different rats.

In the first experiment (test 1), groups of tendons (n=7) were placed in a control solution or an experimental solution. The control solution was commercially prepared phosphate buffered saline (PBS 0.8%NaCl, 0.02%KCl, 0.012%KH2PO4, 0.091%NaHPO4), pH 7.4, to which 0.03%NaN3 had been added. The experimental solution was 1mM NKISK. The two tendon groups were allowed to soak in their respective solutions for 1.5 to 3 hours at 4°C. After soaking, the tendons were pulled to failure at a rate of 0.01mm/sec on a servo-hydraulic materials testing machine. In the second experiment (test 2), two tendon groups (n=9) were marked twice, at a gage length of 15mm near the center of the tendon segment with India Ink. The tendons were inserted individually into glass tubes filled with either the control PBS solution or the experimental 1mM NKISK solution and suspended by the upper end of the tendon. The distance between the gage marks was measured using 1.5X magnification and a micro caliper in a blinded fashion. Each measurement was repeated. If the measurements were within 0.2 mm they were averaged and entered as a result. If not within 0.2 mm, they were discarded and two more measurements were made. The percent creep was calculated at selected time intervals. When the tendons had sustained at least 8% but no more than 12.5% creep they were removed from the apparatus and pulled to failure on the material testing system. Finally, a third experiment was performed (test 3). Two tendon groups (n=9) were prepared in the same manner as in experiment two. In this experiment, however, the tendons were hung in the apparatus for six hours, regardless of the percentage creep sustained.

All tendons were pulled to failure while submerged in PBS solution and then cut free at the grips of the material testing machine. The tendons were allowed to dry for three days. The cross-sectional area was determined based on the dry weight, original grip to grip length, and the specific gravity of rat tail tendon to give the ultimate strength in MPa (3). A mean ultimate strength and standard deviation were calculated for each group. Other parameters such as energy to break point, strain at maximum load, and elastic modulus were also calculated. Significance was determined by the Students t-test, p<0.05.

Results

From test 1, there was a statistically significant difference (p<0.05) in the ultimate strength between the control PBS treated tendons (155.5 MPa, SD=19.7 MPa) and the NKISK treated tendons (111.4 MPa, SD=46.6MPa) after soaking. In test 2, there was no difference (p>0.05) in the ultimate strength when both the control PBS tendons (61.7 MPa, SD=33.6MPa) and the NKISK treated tendons (55.5 MPa, SD=30.6MPa) were allowed the same percentage of creep (mean creep for PBS treated tendons 9.7%, SD=1.3%). Mean creep for NKISK treated tendons 10.0%, SD=1.4%). In the third experiment, there was a statistically significant difference (p<0.05) in the ultimate strength between the control PBS tendons (167.1 MPa, SD=45.1 MPa) and the NKISK treated tendons (94.9 MPa, SD=39.6 MPa) when both groups were allowed to hang for exactly six hours (see Figure 1). There was also a statistically significant difference (p<0.05) in the percentage of creep between the control PBS group (3.5%, SD=1.4%) and the NKISK treated group (8.4%, SD=2.6%). For all three experiments, there were no differences in other parameters such as energy to break point, strain at maximum load, and elastic modulus.

Discussion

It must be noted that a high degree of variability exists between the mechanical properties of different rat’s tail tendons, a limitation to research in this area, which makes it impossible to compare control groups in the three tests. Nonetheless, the findings from this study show that NKISK causes rat tail tendon weakness. More importantly, NKISK induces a higher rate of rat tail tendon lengthening (creep) under constant low stress. These findings are presumably due to competitive inhibition of the interfibrillar bonds between adjacent collagen fibrils within the tendon (1). Interestingly, NKISK does not seem to cause more weakness in rat tail tendons when compared to non treated tendons that have had a similar creep lengthening (though it took 50% more time to reach this similar percent creep in non treated tendons). When tendons were allowed to creep for a similar period of time (with greater creep seen in NKISK treated tendons) the NKISK treated tendons were significantly weaker. This suggests that the weakness found in rat tail tendons might also be dependent upon the amount of overlap remaining between adjacent, discontinuous collagen fibrils. Disruption of this bond by NKISK facilitates higher rates of creep lengthening and ultimately reduces tendon strength.

Acknowledgments

1. National Institute of Health: Short term Research Grant, Award # 2-T35-DK07386-17.

References

3. Torp S. et al. Structure of Fibrous Biopolymers, pp. 197-221, 1975