A PENTAPEPTIDE WHICH INHIBITS DECORIN/FIBRONECTIN BINDING POTENTIATES TENDON STRAIN DURING TENSILE STRESS, PRESUMABLY BY ALLOWING COLLAGEN FIBER SLIDING

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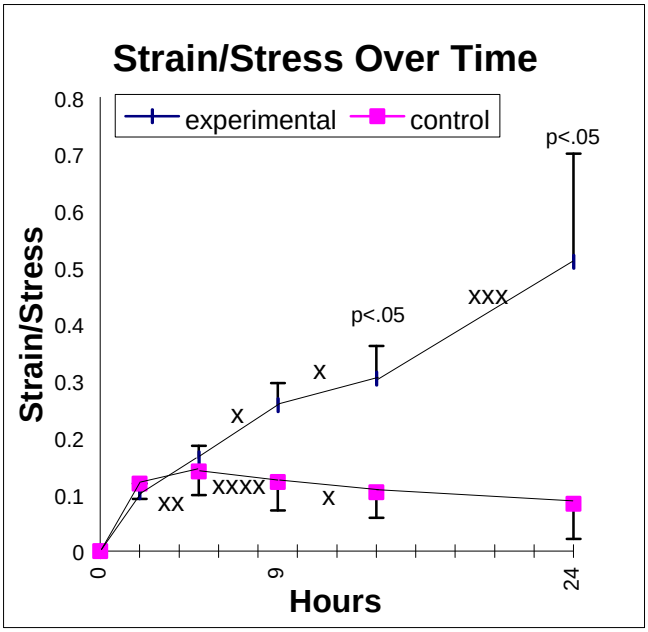
Introduction:
Our research has focused on the phenomenon of length changes in dense collagenous tissues (tendon and ligament) during growth and contracture. We have hypothesized that these length changes occur through the sliding of discontinuous collagen fibrils[6] or fibers past one another. Previous growth and contracture studies from our laboratory have documented diffuse changes in ligament length through marking suture studies[2,7] as well as the movement of marked fibers past one another[8] (phenomena that are compatible with the sliding fibril hypothesis). For fibril sliding to be a viable mechanism there must be a reversible mechanism of fiber to fiber bonding which can be released to allow fiber sliding and then reattached. We have explored this possiblity by subjecting rat tail tendons to tensile stress while they are immersed in various solutions which we have postulated might modify the putative interfibrillar bonds. We initially reported the finding of increased strain in these stressed tendons when immersed in gentamicin, an antibiotic which is a small polycation[3]. Similar increased strains occured with tripeptide polylysine, which has a simlar size and charge configuration to gentamicin. Schmidt, et al has reported that the pentapeptide NKISK (which has two lysine residues and corresponds to the 85-89 sequence of the decorin core protein) inhibits the high affinity interaction between decorin and fibronectin in a charge independent manner.[4] Hedbom and Heinegard have proposed that one possible function of decorin is to connect neighboring collagen fibrils[1]. We therefore hypothesized that this NKISK binding site might play an important role in interfibrillar bonding.

Methods:
The University of North Carolina Peptide Synthesis Facility synthesized the pentapeptide NKISK specifically for these experiments with free amino and carboxy termnii. Phosphate buffered saline pH 7.4 with 0.03%NaN_3 was used as the control immersion solution. For the experimental solution, NKISK was added to a concentration of 0.8mM (the pH was readjusted back to 7.4 with NaOH). Under a dissecting microscope, 6 cm long tendons were pulled from fresh frozen cadaveric (sacrificed for unrelated IRB approved experiments) rat tails and laid over a petri dish cover marked at 15mm intervals. Care was taken to try to select similarly sized, moderately large, apparently single tendons. The tendons were marked at the 15mm intervals with a needle dipped in India ink and then pulled into 5.5mm (i.d.) glass tubes which were plugged at the bottom end and sealed with silicone grease. The tendons were suspended from clips and the tubes filled with experimental or control solutions so that the weight of the tendon, tube, grease, plug and solution all depended from the tendon, subjecting it to a constant load (this assembly was weighed at the end of the experiment in order to determine the load). The distance between the marks on the tendon were measured with a caliper accurate to 0.1mm at the beginning of the suspension period and again after 2, 5, 9, 14, and 24 hrs. At the end of the experiment the portion of the tendon between the marks was air dried three days and then weighed in order to back calculate the cross sectional area of the tendon[5]. The resultant data was analysed for significant differences with the Mann-Whitney rank sum test.

Results:
Although in perfecting the technique several experiments were done with similar results, there is significant tail to tail variability so we report here the results of a single experiment using 22 tendons from a single tail, 11 in each group. For these two groups the mean tendon loads were 0.033 (SD=0.001) N for both groups. The mean cross sectional area was 0.07 (SD=0.03) mm^2 for both groups. Because of the variations in tendon size evident in the tendon cross sectional area SD there was variation in tendon stress ranging from 0.24 to 1.06Mpa. In order to try to control for higher stresses causing faster tendon strain we have plotted Strain/Stress at each time point in the graph.



Legend: Strain/Stress at each time point . "x's" denote tendons which broke between time points. Bars = 1SE.

Discussion:
Tendons exposed to NKISK stretched significantly more than control tendons by the 14hr time point. One experimental tendon reached 40% strain before breaking! The experimental tendons appeared to stretch in a linear fashion with time while you will note that the mean length of the control tendons actually decreased after 5 hrs. A portion of this decrease is due to breakage of high stress tendons which had lengthened more than the remaining low stress tendons but, a portion is also due to the fact that some control tendons actually shortened slightly towards the end of the experiment. Tendon breakage tended to occur quite a bit earlier in the control tendons than in the experimental tendons as if NKISK was protective. One could imagine such protection resulting from the sliding of high stress fibers and thus equalization of stress across the tendon rather than early breakage of high stress fibers which would transfer load to the remaining fibers.
We believe that this evidence supports the proposed role of decorin in interfibrillar bonding[1].as well as the sliding fiber/fibril hypothesis of growth/contracture in dense collagenous tissues.

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