Poly-N-Acetyl Glucosamine (sNAG) is Dose Dependent for Healing of a Rat Rotator Cuff

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Introduction

Rotator cuff injuries are a common musculoskeletal problem and frequently require surgical intervention, with repair failure remaining a frequent problem.[1] Many biologic therapies have been utilized in an effort to improve tendon repair.[2] Our previous work demonstrated that 0.2 mg (one 4 mm round) of Talymed (Marine Polymer Technologies, Inc.) material improved tendon-to-bone healing, with treated supraspinatus tendons demonstrating increased maximum load and maximum stress at 4 weeks post-injury compared to saline-treated controls.[3] However, whether an increased dose of this nanofiber material could further improve tendon-to-bone healing after supraspinatus injury is unknown. Therefore, the purpose of this study was to continue to investigate the healing properties of sNAG polymer in a rat rotator cuff repair model, increasing the dose of Talymed (sNAG) delivered at the site of injury and repair. We hypothesized that this increased dose sNAG would improve supraspinatus tendon-to-bone healing compared to saline-treated controls.

Methods

Study Design

36 adult male Sprague-Dawley rats (400-450g) were used in this IACUC-approved study. All animals underwent bilateral, full thickness transection and repair of the supraspinatus tendon as described.[4-5] Animals were randomized into one of two groups receiving either sNAG or a saline injection (n = 18/group). For sNAG treated animals, immediately prior to repairing the supraspinatus, a 0.8 mg dose of the thin sNAG membrane (4 stacked pieces, 4mm diameter) was placed on the "foot print" of the supraspinatus tendon to bone attachment site. All animals were allowed normal cage activity after surgery. Animals were sacrificed either 2 (n = 6/group) or 4 weeks (n = 12/group) post-injury and repair. Animals sacrificed at 4 weeks underwent longitudinal in vivo ambulatory assessment with measurements pre-injury and 1, 2, and 4 weeks post-injury and repair.[6] *Ex-Vivo*: The right supraspinatus tendons of animals sacrificed at 2 weeks were immediately harvested and processed for histological analysis including quantitative collagen fiber organization analysis.[5,8-9] Animals sacrificed at 4 weeks had their right supraspinatus immediately dissected and processed for histology (n = 6/group) and were frozen at −20°C and later thawed for dissection at the time of quasistatic mechanical testing (n = 12/group).[7-8]

Statistics

Mechanical testing and collagen fiber organization data were evaluated using one-tailed t-tests after confirming data normality. Semi-quantitative histological comparisons were made using Mann-Whitney U tests. Ambulatory assessment comparisons were made using a 2-way ANOVA with repeated measures on time with follow-up t-tests between groups at each time point. Significance was set at $p < 0.05$ for all comparisons.

Results

Mechanical Properties

At 4 weeks after injury, there were no differences between saline-treated control and sNAG-treated tendons for cross-sectional area, maximum load, modulus, or stiffness (Figure 1).

Histologic Observations

Semi-quantitative grading indicated that cellularity was increased with sNAG treatment at the insertion at 4 weeks post-injury (Figure 2A) and in the midsubstance at 2 weeks post-injury (Figure 2B). There were no differences between groups for cell shape in the tendon insertion or midsubstance (Figure 2 C,D).

Ambulatory Measurements

sNAG had no effect at any time point on animal stride width, stride length, stance time, rate of loading, propulsion force, or peak vertical force (Figure 3).

Discussion

The purpose of this study was to further investigate the healing properties of an increased dose of sNAG polymer in a rat rotator cuff repair model. Surprisingly, a higher dose did not

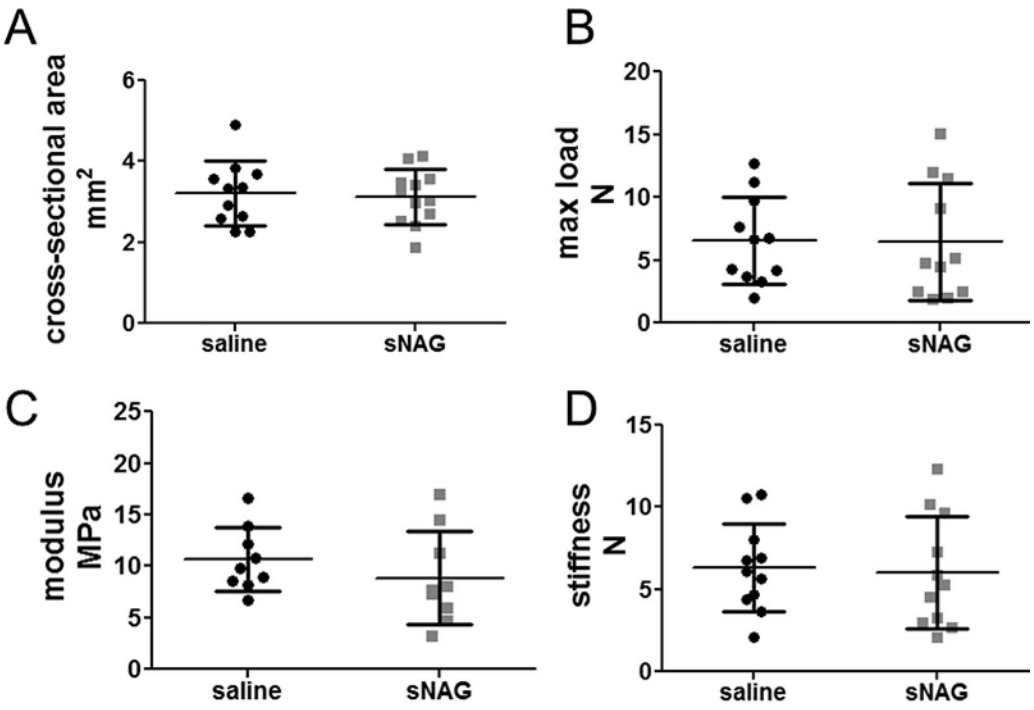

Figure 1. Mechanical Properties. Four weeks after injury, there were no differences between saline-treated controls and sNAG-treated tendons for **(A)** cross-sectional area; **(B)** maximum load; **(C)** modulus; or **(D)** stiffness. Data shown as mean ± SD.

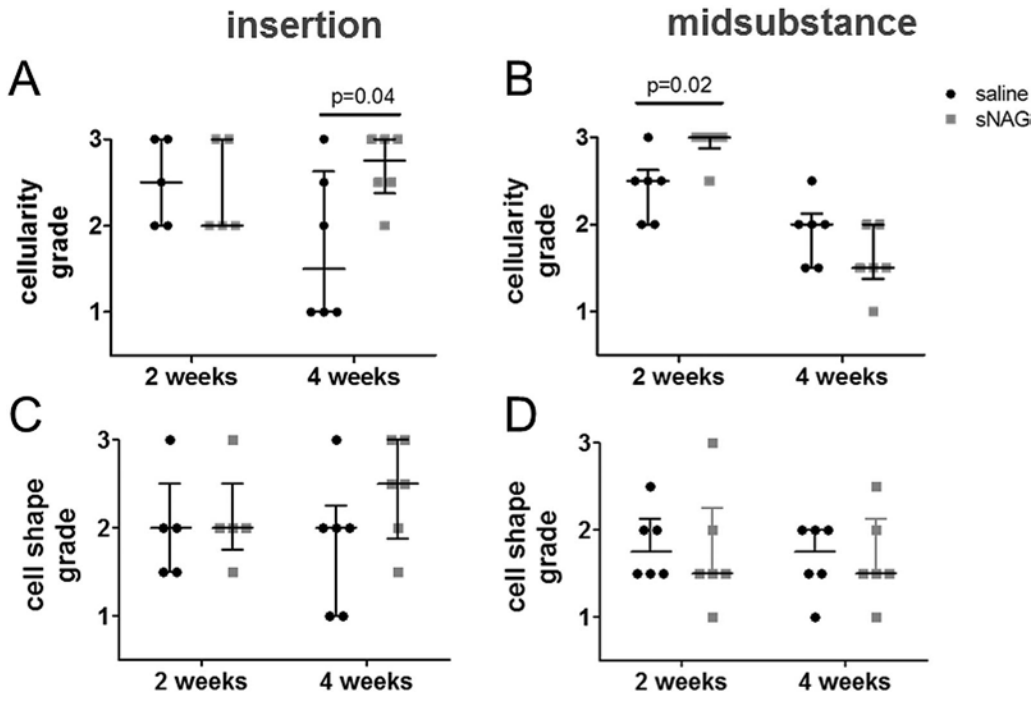

Figure 2. Histological Properties. In the tendon insertion, **(A)** cellularity was increased with sNAG treatment at 4 weeks post-injury. Similarly, in the midsubstance, **(B)** sNAG increased cellularity at 2 weeks. There were no differences between groups for cell shape **(C)** in the tendon insertion or **(D)** midsubstance. Data represented as median ± IQR.

produce significant improvements in rotator cuff healing as was seen with a previously studied "standard dose".[3] Although many parameters did not show differences between groups, there were no negative effects with an increased dose of sNAG. Treated tendons did demonstrate increased tendon cellularity, but this did not translate to improved mechanical properties. Another previous study utilizing repeated injections of 0.2 mg of a liquid-formulation of sNAG led to improved Achilles tendon healing 3 weeks after a full thickness, partial width tear.[10] This study suggests that sNAG may be dose-dependent. Early histological changes with this dose could lead to later improvements in tendon strength; further studies are needed to investigate this possibility, as well as to explain the mechanism of action for the changes identified.

Significance

The effects that sNAG has on rotator cuff tendon healing may be dose-dependent, as the higher dose tested in this study (4x original) did not improve tendon properties.

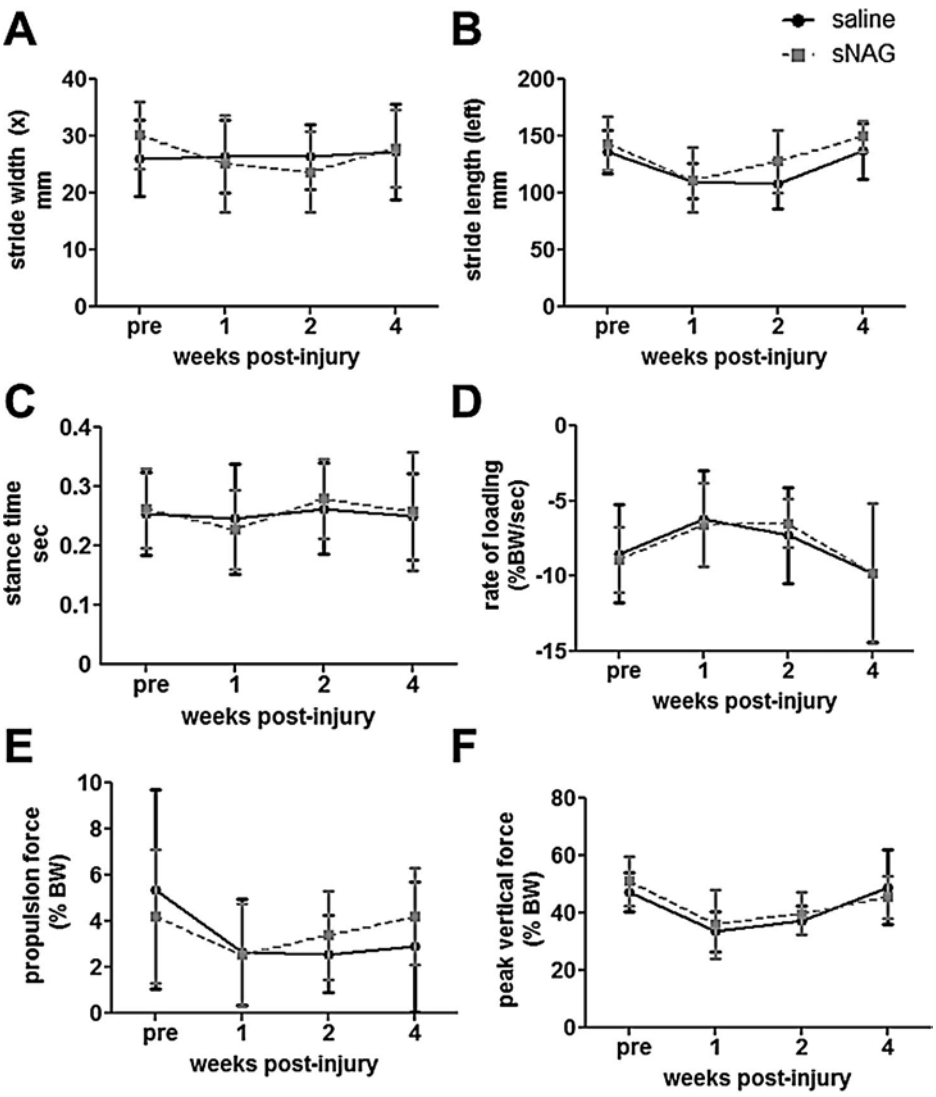

Figure 3. Gait Properties. sNAG treatment had no effect at any time point on animal **(A)** stride width between paws; **(B)** stride length; **(C)** stance time; **(D)** rate of loading; **(E)** propulsion force; or **(F)** peak vertical force. Data shown as mean ± SD.

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Disclosures

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