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Poly-N-Acetyl Glucosamine (sNAG) Enhances Rotator Cuff Tendon Healing in a Rat Model

Courtney Nuss[1]
Daniel Choi, BS[1]
Julianne Huegel, PhD[1]
Stephanie Weiss, BS[1]
John Vournakis[2]
Louis Soslowsky, PhD[1]

[1]McKay Orthopaedic Research Laboratory, Philadelphia, PA

[2]Marine Polymer Technologies, Inc., Burlington, MA

Introduction

Rotator cuff injuries are a common musculoskeletal problem frequently requiring surgical intervention which, unfortunately, has a high failure rate[1]. Various forms of biological augmentation have been utilized in an attempt to improve tendon repair[2]. Poly-N-acetyl glucosamine (sNAG) polymer containing nanofibers have been shown to increase the rate of healing of venous leg ulcers, with an 86% success rate clinically[3]. However, whether this nanofiber material could improve tendon-to-bone healing is unknown. Therefore, the purpose of this study was to investigate the healing and potential analgesic properties of sNAG containing membranes in a rat rotator cuff tendon injury and repair model. We hypothesized that sNAG would improve tendon-to-bone healing and reduce pain.

Materials & Methods

Study Design

80 adult male Sprague-Dawley rats (400-450g) were used in this IACUC-approved study. All animals underwent a full thickness transection and repair of the left supraspinatus tendon as described[4,5]. Before repairing the supraspinatus in half the animals, a thin membrane of sNAG (4mm diameter) was placed on the "foot print" of the supraspinatus tendon to bone insertion. Animals were further subdivided, receiving only 1 day of analgesics (buprenorphine) on the day of surgery or receiving the standard 3 days of analgesics. Therefore, animals were randomized into one of four groups receiving: 1) only 1 day of analgesics, 2) the standard 3 days of analgesics, 3) 1 day of analgesics with sNAG, and 4) 3 days of analgesics with sNAG. All groups were allowed normal cage activity after surgery. Animals were sacrificed either 2 (n = 4 per group) or 4 weeks (n = 16 per group) post-injury and repair. Animals sacrificed at 4 weeks underwent a longitudinal in vivo ambulatory assessment with measurements 1 day pre-injury and 3,7,14, and 28 days post-injury and repair[6].

Ex-Vivo

The 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 were either immediately dissected and processed for histology (n = 4 per group), or frozen at -20°C and later thawed for dissection at the time of quasistatic mechanical testing[7,8] (n = 12 per group).

Statistics

Mechanical testing and collagen fiber organization data were evaluated using two-tailed t-tests. 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 tests.

Results

Mechanical properties

Mechanical property differences were observed between groups with and without sNAG that received 3 days of analgesics. Specifically, in the presence of analgesics, tendons receiving the sNAG polymer had significantly increased max load and max stress (Figure 1A, B), as compared to tendons without sNAG. No mechanical differences were observed between groups treated with and without sNAG when only 1 day of analgesics was provided. Additionally, the use of analgesics alone did not have an effect on mechanical properties.

Histologic observations

Several differences were seen between groups receiving 3 days of analgesics. At 4 weeks, cellularity was increased at both the insertion and midsubstance in non-sNAG animals compared to those treated with sNAG. Cells were more round at 2 weeks in the midsubstance and at 4 weeks in the insertion in sNAG treated animals compared to the non-sNAG animals. No significant differences were seen in collagen organization or other histological parameters.

Ambulatory Measurements

No differences were observed in ambulatory measurements between groups treated with 1 and 3 days of analgesics in the absence of sNAG. Improvements were observed at 14 days

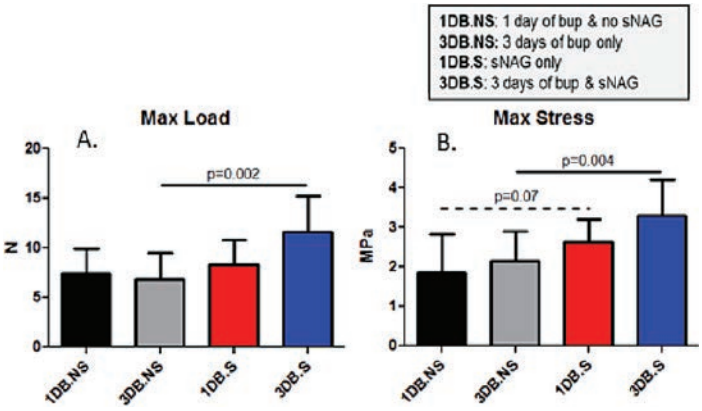

Figure 1. Both max load **(A)** and max stress **(B)** are significantly increased in the supraspinatus of animals treated with sNAG compared to non-sNAG in the presence of the standard 3 days of analgesics (buprenorphine.)

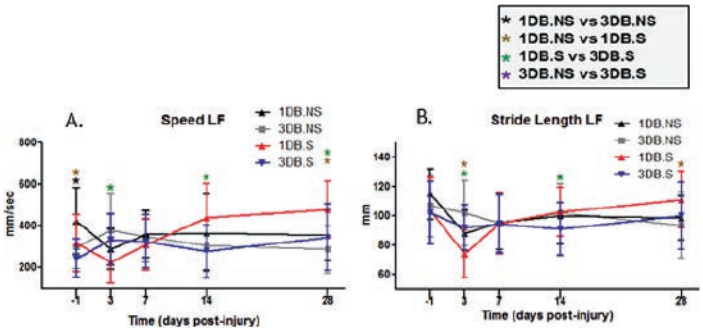

Figure 2. Speed **(A)** and stride length **(B)** of the left front limb (LF) At three days both parameters are significantly decreased in animals treated with sNAG and one day of analgesics (1DB.S) compared to those treated with and three days of analgesics (3DB.S.) However, these parameters are improved at 14 and 28 days (comparison indicated with green asterisk.)

in stride length in animals treated with sNAG compared to animals without sNAG with 1 day of analgesics. Importantly, differences were observed in ambulatory measurements in animals treated with sNAG and receiving either 1 or 3 days of analgesics. Animals receiving only 1 day of analgesics showed decreased speed and stride length at 3 days post-injury and repair, but showed increases in the same parameters at 14 days post-injury and repair (Figure 2A,B).

Discussion

Animals treated with sNAG showed increase in max load and max stress (Figure 1A, B) suggesting that the presence of this polymer improves mechanical strength of the repaired tendon-to-bone healing construct. No other mechanical differences were observed between groups treated with and without sNAG with 1 day of analgesics. Therefore, the observed ambulatory differences in these groups could be associated with pain rather than a structural deficit. For those given 3 days of analgesic, mechanical differences were observed between groups treated with and without sNAG. Therefore, the observed ambulatory differences in these groups could be a result of both pain and structural deficit. When comparing animals treated with sNAG but received differed time courses of analgesics, ambulatory measurements showed decreased speed and stride length at 3 days post injury and repair with only 1 day of analgesic, but improvement in speed and stride length at 14 and 28 days in the same group. This may be explained by the rationale that animals receiving analgesia for a shorter time may experience pain early in healing, limiting weight-bearing on their injured limb. This decreased loading may have led to modest long term functional improvements as well as improved tendon-to-bone healing[10]. Alternatively, those who received the standard 3 days of analgesics supposedly felt less pain, and therefore bore more weight, resulting in delayed improvements in functionality, as seen at 14 and 28 days postop. Because animals in this study were evaluated at relatively early time points, the long-term outcome of the use of sNAG is not fully known. Future studies should evaluate healing at longer time points. Additionally, dosage studies may identify a more effective quantity of sNAG for tendon-to-bone healing. Finally, studies to elucidate the mechanism of action for the changes identified are important.

Conclusions

sNAG improves tendon-to-bone healing in a rat rotator cuff detachment and repair model with the potential of long-term analgesic effects. These results support further study to understand the long-term effects, as well as mechanism of action, of sNAG on tendon healing and analgesia.

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Disclosures

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