The Role of Lubricin in Intrasynovial Tendon Gliding

INTRODUCTION:
Lubricin is a mucinous glycoprotein originally isolated from synovial fluid, and noted to articular cartilage. Lubricin has been identified on the flexor tendon surface and shown to play an important role in tendon lubrication. Recently we compared tendon fascicle gliding resistance in wild type and lubricin knockout mice and found the absence of lubricin increased tail tendon fascicle gliding resistance. Based on this study we hypothesized that the absence of lubricin would increase gliding resistance of intrasynovial tendons. The purpose of this study was to investigate the role of lubricin in intrasynovial tendons by comparing gliding resistance in lubricin knockout, heterozygous, and wild type mice.

MATERIALS AND METHODS:
Preparation of Specimen for Measurement of Tendon Gliding Resistance: Thirty-six hind paws were obtained from eighteen adult mice, six lubricin knockout mice (lubricin –/–), six heterozygous mice (lubricin +/-), and six wild type mice (lubricin +/+). A total of thirty-six flexor digitorum longus (FDL) tendons in the third digits of each hind paw were used. In each harvested paw, a longitudinal incision was made and the flexor sheath, including the distal pulley, was removed. The tendon was marked at the proximal edge of the pulley. The tendon for each edge of tendon and then the tendon was cut at the outside of each loop. (Figure 1)

Measurement of Tendon Gliding Resistance: Each paw was fix on a flat platform with the third digit kept in full extension in a saline bath. The backside of the paw was affixed with cyanoacrylate adhesive and the digit was held with small clip. A 150-g load transducer (GS0-150, Transducer Techniques, Temecula, CA) was connected to the proximal end of the FDL tendon. A 5-g weight and cord was connected to the distal end of the tendon and passed over a low-friction mechanical pulley to maintain tension on the FDL tendon. The proximal load transducer (F) was connected to a custom-made mechanical actuator with a small linear slide driven by a precision gear head stepper motor controlled by a motor driver/microcontroller (ACE-SDE, Arcus Technology, Livermore, CA). Based on the experience of previous studies, a set arc of contact, 30° and 20° between the horizontal plane and the proximal transducer and distal cable extension, respectively, was used to measure the gliding resistance (Figure 2). The tendon was pulled proximally by the actuator at a rate of 0.25 mm/s. Maximum excursion was set at 4 mm. The force at the proximal tendon end and the corresponding displacement was recorded at a sampling rate of 20 Hz. The friction load shows a distinct plateau once the tendon begins sliding. It was assumed that the load generated by the mass remained nearly constant. The gliding resistance, as defined and as an average of friction forces evaluated in flexion and extension, can be obtained from the following equation:

\[ F_{G} = \text{mean}(F_{\text{plateau,flexion}} - F_{\text{plateau,extension}})/2 \]

It can further be demonstrated that the total gliding resistance can be corrected for the mechanical pulley friction to obtain the friction between the tendon and pulley as follows:

\[ F_{G} = F_{G,\text{total}} - F_{G,\text{pulley}} \]

RESULTS:

Measurement of Tendon Gliding Resistance: The gliding resistance of the lubricin knockout mice was significantly higher than the wild type and heterozygous mice. There was no significant difference in gliding resistance between wild type and heterozygous mice (Figure 3).

Histology: From each group, three FDL tendon segments and tendon sheaths, including the proximal pulley, were fixed in 4% paraformaldehyde buffer solution. After fixation the samples were decalcified with Decalcifying Solution B (Wako, Japan) and embedded in Tissue-Tek O.C.T. Compound (Sakura Finetek, Inc, CA). Sections 15 μm thick were cut in the transverse plane using a Leica microtome (Leica Microsystems, Wetzlar, Germany). The sections were stained with hematoxylin and eosin (H&E), and then mounted on glass slides. The morphology and cellularity were evaluated with light microscopy.

Scanning Electronic Microscopy (SEM): From each group, three FDL tendons were prepared for scanning electron microscopy (SEM). The selected tendons were fixed in a solution of buffered glutaraldehyde and osmium tetroxide. After dehydration in graded acetone, the specimens were coated with gold/palladium alloy and examined by SEM (Hitachi S-4700, Hitachi, Japan) in secondary electron mode at 3kV. The tendon surface was qualitatively assessed for smoothness.

Statistical Analysis: The average of the gliding resistance was analyzed using one-way analysis of variance (ANOVA) followed by the Tukey post hoc test. A P-value of 0.05 or less indicates a significant difference between groups.

DISCUSSION:
In this study we developed a new system that is able to assess gliding resistance in small tendons. Using this system we found that the FDL gliding resistance increased in lubricin knockout mice compared to wild type and heterozygous mice. We also found some histological and structural changes in the lubricin knockout tendons. Our findings confirm the importance of lubricin in the lubrication of intrasynovial tendon.

SIGNIFICANCE:
A lack of lubricin is associated with poorer tendon gliding function. This knockout model may be useful in determining the effect of lubricin on tendon healing and the response to injury.

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