Prevention of Cartilage Degeneration and Gait Asymmetry by Lubricin Tribosupplementation in the Rat Following Anterior Cruciate Ligament Transection

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Objective. To investigate whether cartilage degeneration is prevented or minimized in a rat model of anterior cruciate ligament (ACL) injury following a single dose-escalated intraarticular injection of lubricin derived from human synoviocytes in culture.

Methods. Unilateral ACL transection (ACLT) of the right hind limb was performed in Lewis rats (n = 56). Control animals underwent a capsulotomy alone, leaving the ACL intact (n = 11). Intraarticular injections (50 μl/injection) of phosphate buffered saline (PBS; n = 14 rats) and human synoviocyte lubricin (1,600 μg/ml; n = 14 rats) were performed on day 7 postsurgery. Animals were killed on day 70 postsurgery. Histologic specimens were immunoprobed for lubricin and sulfated glycosaminoglycans. Urinary C-telopeptide of type II collagen (CTX-II) levels were measured on days 35 and 70 after surgery. Hind limb maximum applied force was determined using a variable resistor walkway to monitor quadruped gait asymmetries.

Results. Increased immunostaining for lubricin in the superficial zone and on the surface of cartilage was observed in lubricin-treated and control animals but not in PBS-treated or untreated animals with ACLT. On days 35 and 70 after surgery, urinary CTX-II levels in human synoviocyte lubricin–treated animals were lower than in untreated and PBS-treated animals (P < 0.005 and P < 0.001, respectively). Animals with ACLT treated with human synoviocyte lubricin and control animals distributed their weight equally between hind limbs compared to PBS-treated or untreated animals (P < 0.01).

Conclusion. Our findings indicate that a single intraarticular injection of concentrated lubricin following ACLT reduces type II collagen degradation and improves weight bearing in the affected rat joint. These findings support the practice of tribosupplementation with lubricin for retarding cartilage degeneration and possibly the development of posttraumatic osteoarthritis.

Acute anterior cruciate ligament (ACL) injury is a significant risk factor for the development of posttraumatic osteoarthritis (OA) (1,2). Contributing factors, such as joint instability, altered joint loading (3,4), enzymatic tissue degradation (5,6), and a lack of lubrication (7), are postulated to play a significant role in the pathogenesis of OA following injury. Synovial fluid (SF) lubricin concentrations in the injured joints of patients with ACL injury were found to be significantly lower than those in the uninjured contralateral joints (7).

Inhibition of tumor necrosis factor α (TNFα) with etanercept in a rat model of ACL transection (ACLT) was shown to up-regulate lubricin expression, reduce friction, and reduce sulfated glycosaminoglycan (sGAG) loss from cartilage (8). However, the translational value of inhibiting TNFα in preserving chondro-protection may be limited by confounding side effects related to immune system dysregulation. Thus, the direct intraarticular reintroduction of lubricin (i.e., tribosupplementation), during the peri-injury period may offer
an opportunity to preserve cartilage by re-establishing a protective covering of lubricin. This approach appears effective in the rat meniscectomy model using lubricin with a truncated mucin domain (9). Disease-modifying effects were noted using 3 injections per week, which may have ameliorated pain (10). Lubricin was also effective in the rat model of ACLT on day 35 after surgery when human SF lubricin, human synoviocyte lubricin, and human recombinant lubricin were tested (11).

ACLT rupture is a common injury, resulting in >250,000 surgical reconstructions per year in the US. These injuries place the knee at risk for early posttraumatic OA despite surgical treatment (12). The present study was designed to determine whether a single lubricin dose-escalated treatment, as compared to no treatment or phosphate buffered saline (PBS) treatment as a placebo, could replicate the chondroprotective effects observed previously (11). We hypothesized that rats with ACLT (13) treated with lubricin would show less gait asymmetry and fewer degradation products of urinary C-telopeptide of type II collagen (CTX-II) at 10 weeks than their untreated and placebo-treated counterparts. We also posited that there would be less radiographic evidence of OA, and evidence of GAG and lubricin neosynthesis, which was observed earlier following 7 separate intraarticular injections of lubricin (11). The lubricin we used, human synoviocyte lubricin, was purified from synoviocytes in culture, and is a proxy for full-length human recombinant lubricin (11).

MATERIALS AND METHODS

Animal model of ACLT. ACLT was performed on the right knee joints of 8–10-week-old male Lewis rats. A total of 56 animals were subjected to surgery, and they were randomly assigned, in equal numbers, to 1 of the following 4 treatment groups: ACLT plus human synoviocyte lubricin, ACLT plus PBS (placebo treatment), untreated ACLT (no injection), or capsulotomy (sham operation and no injection), where the right knee synovium was incised and then closed with polydioxanone suture. Animals were anesthetized with ketamine and medetomidine, the skin was prepped with a topical antiseptic, and an incision was made in the skin laterally to the right knee joint. After the joint capsules were opened, the ACL was transected using a surgical hook and scalpel. In all animals, the right knee joint was the operated joint and the left knee joint was the ACL-intact contralateral joint. Prior studies (8,14) have established that the contralateral joints do not display pathology and that they appear normal; thus, they were not included in the analyses. A total of 5 animals died during surgery; these were from the untreated ACLT and capsulotomy groups (leaving 12 animals in the untreated ACLT group and 11 animals in the capsulotomy group). All animals were killed on day 70 after surgery, and anteroposterior and lateral radiographs of each knee were obtained using a high-resolution (14-bit) radiography system (MX-20; Faxitron) (15). Afterward, the right knee joints were placed in neutral buffered formalin and paraffin embedded. The study was approved by the Institutional Animal Care and Use Committee. Surgeries and injections were conducted by investigators (KAK and SCA) who had extensive experience in performing and replicating this procedure. Injections were performed by 2 additional investigators (KAE and KW).

Lubricin. Lyophilized human synoviocyte lubricin was obtained from SBH Sciences and reconstituted in PBS at pH 7.4 at a concentration of 1.6 mg/ml. Human synoviocyte lubricin was purified from conditioned medium from human synoviocytes growing confluent. The purification procedure was similar to one previously described (16). According to the manufacturer, this molecule is antiadhesive, as tested in a cell-based assay (17), and lubricates a cartilage-against-cartilage bearing assay (18). Sequence confirmation of PRG4 was obtained by liquid chromatography and mass spectrometry of a tryptic digest of purified human synoviocyte lubricin.

Lubricin dosing. Seven days after ACLT, animals were anesthetized with inhaled isoflurane and were injected intraarticularly with 80 μg human synoviocyte lubricin in a 50-μl volume (266 μg/kg per animal). Injections were administered into the patellar tendon of the operated rat knee joint each time while the knee was flexed. Confirmation of intraarticular administration was confirmed by a noticeable and palpable SF collection.

Quadruped gait analysis. A pressure-sensing walkway measuring 5 inches wide by 14 inches long (Tekscan) was used to monitor paw pressure and impulse (Newton-seconds) over 2 gait cycles with a paw strike resolution of 15.5 sensels/cm² on day 70 after surgery, just before animals were killed. The walkway was first calibrated with known weights which occupied similar number of sensels as would a rat paw, and the weight of each animal in Newtons was calculated and entered into the Tekscan operating system software (version 7.0) prior to data collection. Data files consisted of consecutive images (frames) sampled at 200 Hz, which showed quadruped progression wherein hind limb strikes superimpose upon fore limb strikes. Paw pressure distribution is illustrated with isobars (Figure 1). The maximum paw strike force of the hind limbs and the hind limbs and stance time of each gait cycle and within each walkway trial were determined. From this, strike boxes were calculated (Figure 1) within Tekscan software, which calculated the center of symmetry between the fore limbs and hind limbs and their trajectory. From this parameter, the differential weight bearing of the left versus right hind limb was calculated across gait cycles and expressed as a ratio of the average maximum force of the left hind limb divided by the average maximum force of the right hind limb. This system has been used in gait studies in previous investigations in small mammals (19–21).

Determination of urinary CTX-II concentrations in untreated, PBS-treated, and human synoviocyte lubricin–treated animals with ACLT. On days 35 and 70 after surgery, animals were housed in metabolic cages, and 24-hour urine samples were obtained. Urine samples were subsequently centrifuged at 5,000 rpm for 20 minutes and stored at −20°C. Urinary CTX-II concentrations were determined using the Pre-Clinical Urine CartiLaps enzyme-linked immunosorben
Urinary creatinine was determined using the QuantiChrom creatinine assay (Bioassay Systems). Urinary CTX-II levels were normalized to urinary creatinine. Due to the availability of a limited number of metabolic cages, urine was collected from 12 rats with untreated ACLT, 14 rats with ACLT treated with human synoviocyte lubricin, 14 rats with ACLT treated with PBS, and 6 capsulotomized rats.

Histologic analysis and immunostaining. Paraffin-embedded coronal sections were obtained from weight-bearing areas of the articular cartilage of joints with ACLT from each animal as previously described (11). Microtomed coronal sections were collected every 250 μm in order to find a representative area showing femoral condyles, tibial plateaus, and the menisci. Two adjacent sections were collected through this region and stained with Safranin O–methyl green for assessment of cartilage sGAG content. Two additional adjacent sections were immunoprobed for lubricin and GAGs. Specific staining for a glycosylated epitope within the lubricin mucin domain was performed with monoclonal antibody 9G3 (provided by M. Warman, MD, Boston Children's Hospital, Boston, MA) at a 1:100 dilution. Biotinylated anti-mouse IgG at a 1:200 dilution was used and was detected using the Vectastain ABC kit (Vector).

Probing for GAGs was performed at a 1:100 dilution with monoclonal antibodies 3B3 and 2B6, which are specific for chondroitin 6-sulfate (C6S) and chondroitin 4-sulfate (C4S), respectively (22–24). Biotinylated anti-mouse IgG at a 1:200 dilution was used and was detected using the Vectastain ABC kit (Vector).

Caspase 3 staining. Sections were heated to 60°C for 30 minutes, deparaffinized, and hydrated in xylene and alcohol. Rabbit polyclonal antibody against active caspase 3 (catalog no. ab13847; Abcam) at a 1:50 dilution was added to slides at 4°C overnight according to Vectastain procedures. Following the addition of biotinylated secondary antibody solution and diaminobenzidine, slides were counterstained with 1% methyl green, and coverslip slides were fixed with Permount (Fisher).

TUNEL staining. Decalcified and embedded sections were warmed to 57°C for 5 minutes, deparaffinized by immersing the slides in xylene and ethanol, followed by washing 2 times in 1× PBS, followed by covering sections with proteinase K solution for 20 minutes according to the recommendations of the manufacturer (catalog no. 4812-30-k; Trevigen). After
washed with deionized water, slides were immersed in 1× terminal deoxynucleotidyl transferase (TdT) labeling buffer, and incubated sections were labeled with reaction mixture for 60 minutes at 37°C in a humidity chamber. After using 1× TdT stop buffer to stop the reaction, and 1× PBS wash, slides were incubated with strep-fluor solution for 20 minutes in the dark. After PBS wash and placement of a glass coverslip using a fluorescent mounting medium, slides were viewed under fluorescence microscopy using a 495-nm filter.

**Histologic scoring and image densitometry.** The OA Research Society International (OARS) modified Mankin score (27) was used to measure tibial cartilage degeneration in each rat joint compartment and was averaged. A group of at least 3 scorers who were blinded with regard to treatment group independently graded the best-appearing Safranin O–methyl green-stained section from each rat joint and arrived at a consensus score (11).

**Scoring of rat knee joint radiographs.** Radiographs of the knee joint with ACLT and the contralateral unaffected knee joint in each rat were scored using a modification of the Kellgren/Lawrence scale (28). The Kellgren/Lawrence scale for knee OA as originally described assigns a score from 0 to 4 based on the severity of the disease in human subjects. Although there are some anatomic differences between rats and humans, the images were scored relative to control radiographs of the unoperated contralateral rat limb. Radiographs were scored by an orthopedic surgeon who specializes in adult joint reconstruction and by a fifth-year orthopedic surgery resident. Both examiners were blinded with regard to animal identifier and treatment group when scoring. The respective scores of both examiners for each animal were averaged for analysis.

**Statistical analysis.** The ratio of the average maximum force of the left hind limb to the average maximum force of the right hind limb during gait analyses and the rat urinary CTX-II concentrations are represented by scatter plots and box plots, respectively. The horizontal line within each scatter plot represents the mean, and the outer lines are 1 SD. The horizontal line within each box plot represents the median, and the outer lines are the data minimum and maximum. The mean and 95% confidence interval (95% CI) were calculated for all other dependent variables. Kruskal-Wallis analysis of variance was used to determine differences in the ratio of the average maximum force of the left hind limb to the average maximum force of the right hind limb, urinary CTX-II concentrations, FISH intensity values, OARS scores, and Kellgren/Lawrence scores across treatment groups. P values less than 0.05 were considered significant.

**RESULTS**

**Findings of rat gait analysis.** The rats treated with human synoviocyte lubricin following ACLT demonstrated a mean ratio of average maximum force of the left hind limb to average maximum force of the right hind limb of 0.84 (95% CI 0.73, 0.94), which was significantly lower than that in rats treated with PBS as placebo (1.46 [95% CI 1.11, 1.83]) (P < 0.001) and untreated rats with ACLT (1.08 [95% CI 1.03, 1.14]) (P < 0.01) (Figure 2). Capsulotomized rats with intact ACLs demonstrated a ratio of average maximum force of the left hind limb to average maximum force of the right hind limb of 0.95 (95% CI 0.92, 1.00). There was no significant difference between the capsulotomized (sham) rats and the rats with ACLT that were treated with human synoviocyte lubricin (P = 0.07).

**Urinary CTX-II concentration.** The mean urinary CTX-II levels on days 35 and 70 after surgery were 113.2 pg/mg creatinine (95% CI 106.9, 119.5) and 116.8 pg/mg creatinine (95% CI 110.2, 123.4), respectively, in the human synoviocyte lubricin–treated animals, which were lower than those in the PBS-treated animals at both time points (135.4 pg/mg creatinine [95% CI 123.2, 149.6] in PBS-treated animals on day 35 [P < 0.001] and 157.5 pg/mg creatinine [95% CI 141.9, 173.1] in PBS-treated animals on day 70 [P < 0.001]) (Figure 3). Urinary CTX-II levels in human synoviocyte lubricin–treated animals were higher than those in capsulotomized animals on day 35 (P = 0.002) and day 70 (P = 0.003). Urinary CTX-II levels in human synoviocyte lubricin–treated animals were lower than those in untreated animals with ACLT on day 35 (P = 0.002) and day 70 (P < 0.001). Urinary CTX-II levels trended higher over time in untreated animals with ACLT (P < 0.001). There were no significant differences between
urinary CTX-II levels on day 35 and those on day 70 for capsulotomized animals \((P = 0.671)\), human synoviocyte lubricin–treated animals \((P = 0.698)\), or PBS-treated animals \((P = 0.622)\).

**OARSI histologic grading.** Representative Safranin O–methyl green–stained sections of tibial plateaus from human synoviocyte lubricin–treated animals, PBS-treated animals, untreated animals with ACLT, and capsulotomized control animals are shown in Figure 4. Compared to PBS-treated rat joints, human synoviocyte lubricin–treated rat tibial cartilage typically showed more intense staining that spanned the superficial, middle, and deep cartilage zones, indicating higher sGAG content. Human synoviocyte lubricin–treated rat joints appeared similar to capsulotomized rat cartilage in the intensity of Safranin O staining. Less chondrocyte clon- ing was noted in cartilage from lubricin-treated rat joints than in cartilage from untreated or PBS-treated rat joints. However, the mean OARSI score for human synoviocyte lubricin–treated rat joints \((9.33 [95\% \text{ CI} 7.91, 10.74])\) was not significantly different from that for PBS-treated rat joints \((11.42 [95\% \text{ CI} 9.36, 13.49])\) \((P = 0.18)\) or untreated rat joints \((10.75 [95\% \text{ CI} 6.79, 14.7])\) \((P = 0.57)\). Capsulotomized rat joints had a mean OARSI score of 0.5 \((95\% \text{ CI } -0.17, 1.17)\), which was significantly lower than the scores in the other 3 groups.

**TUNEL staining and GAG immunohistochemistry.** Untreated or PBS-treated joints with ACLT were positive for caspase 3 and were TUNEL positive in the superficial and intermediate zones (Figure 5). Lack of cellular death due to apoptosis, as indicated by absent

\[\text{Figure 3. Urinary C-telopeptide of type II collagen (CTX-II) levels, normalized to creatinine levels, in control rats with capsulotomy, untreated rats with ACLT, rats with ACLT treated with PBS on day 7 after surgery, and rats with ACLT treated with human synoviocyte lubricin on day 7 after surgery. Urine was collected for 24 hours from all animals on days 35 and 70 after surgery. Data are shown as box plots. Each box represents the 25th to 75th percentiles. Lines inside the boxes represent the median. Whiskers represent the data minimum and maximum. All urinary CTX-II assays were performed in duplicate. }\]

\[\text{Figure 4. Safranin O–stained and immunoprobed tibial plateau cartilage from A, rats with capsulotomy, B, untreated rats with ACLT, C, rats with ACLT treated with PBS, and D, rats with ACLT treated with human synoviocyte lubricin. The monoclonal antibodies 2B6 and 3B3 (—) were used to probe for chondroitin 4-sulfate (C4S) and chondroitin 6-sulfate (C6S), respectively. See Figure 2 for other definitions.}\]

\[\text{Figure 5. Immunostaining for lubricin using monoclonal antibody 9G3, fluorescence in situ hybridization (FISH) for lubricin mRNA, caspase 3 staining, and TUNEL staining on day 70 after surgery in the articular cartilage from A, a representative control rat with capsulotomy, B, a representative untreated rat with ACLT, C, a representative rat with ACLT treated with PBS, and D, a representative rat with ACLT treated with human synoviocyte lubricin. Untreated rat joints with ACLT and rat joints with ACLT treated with PBS displayed caspase 3 activity and were TUNEL positive. See Figure 2 for other definitions.}\]
TUNEL staining, was similar in human synoviocyte lubricin–treated rat joints and capsulotomized control rat cartilage. The apparent elevation in GAG content in human synoviocyte lubricin–treated rat joints was supported by greater immunohistochemical staining for 2B6, which is indicative of C4S. By contrast, minimal staining was observed in the untreated or PBS-treated rat joints with ACLT (Figure 4), where counterstaining was readily observable in cartilage. There was no detection of nascent C6S in any of the 4 groups (Figure 4).

**Lubricin immunostaining and FISH.** Rat joints with ACLT that were treated with a single dose of human synoviocyte lubricin and probed with 9G3 showed greater surface staining and the presence of superficial zone chondrocytes that stained positive for lubricin intracellularly (Figure 5D). Control cartilage from capsulotomized (sham-operated) rat joints had a similar appearance (Figure 5A). These findings contrasted with those in a representative PBS-treated rat joint (Figure 5C) and an untreated rat joint with ACLT (Figure 5B), where lubricin immunostaining of the superficial zone and articular surface was absent or minimal, respectively. In situ hybridization indicated that lubricin expression was enhanced following treatment with human synoviocyte lubricin (Figure 5D). The mean IOD for these specimens (496.8 [95% CI 330.6, 662.9]) was significantly greater than for rat joints with ACLT treated with PBS (99.6 [95% CI 53.3, 145.8]) (P < 0.001) (Figure 5C), untreated rat joints with ACLT (215.2 [95% CI 146.8, 283.7]) (P = 0.02) (Figure 5B), or capsulotomized control rat joints (159.3 [95% CI 95.7, 222.9]) (P < 0.001) (Figure 5A).

**Radiography of the rat knee joints.** There were no significant differences between the experimental groups with regard to the Kellgren/Lawrence score. The mean Kellgren/Lawrence scores for untreated rat joints with ACLT and rat joints with ACLT treated with PBS were 2.14 (95% CI 1.56, 2.72) and 1.83 (95% CI 1.49, 2.17), respectively. Rat joints with ACLT treated with lubricin and capsulotomized rat joints had mean scores of 1.89 (95% CI 1.43, 2.35) and 1.33 (95% CI 0.85, 1.81), respectively.

**DISCUSSION**

We have shown in this study and in prior ones (11,15) that some OA degenerative changes following an ACL injury can be mitigated by the intraarticular supplementation of lubricin. Immunohistochemical articular surface staining for lubricin was stronger among the lubricin-treated rat joints in this study. Untreated and placebo treated rat joints with ACLT displayed less surface and minimal superficial zone lubricin, which in a Prg4-mutant mouse model was shown to be critical in the chondroprotection of articular cartilage (29–31). In addition, the human synoviocyte lubricin–treated rat joints with ACLT showed evidence of superficial zone chondrocyte immunopositivity for lubricin, which is related to native lubricin synthesis by superficial and intermediate zone chondrocytes.

Taken together, the findings of lubricin immunohistology and the findings of in situ hybridization, shown in Figure 5, lead us to conclude that, following injury, lubricin expression could be enhanced by the intraarticular delivery of lubricin during the peri-injury period. Without this intervention, loss of lubricin from the cartilage surface occurs due to inflammation and the lack of lubricin expression on superficial zone chondrocytes (8,11). Conversely, control cartilage shows a normal amount of lubricin immunostaining (8,11) and a nominal level of expression, which may be indicative of homeostasis. Introducing lubricin into the traumatized joint protects the superficial zone, appears to prevent cell death, and results in greater C4S expression.

These observations are similar to the findings of our prior lubricin tribosupplementation study (11), in which 2 intraarticular injections were administered per week for a total of 7 injections. However, that study also demonstrated immunoreactivity with 3B3(–), which indicates nascent C6S expression and repair, which was not observed in the present, dose-escalated single-injection study. That previous study also showed an improvement in OARSI scores following lubricin supplementation in the rat, but overall this metric appeared to be insensitive when compared to other data suggesting anabolic or chondroprotective outcomes in the rat joints with ACLT. The present study was limited to a single dose-escalated lubricin intraarticular injection, and, by contrast, no improvement in OARSI scores was observed. This suggests that multiple lubricin injections may be needed to achieve a chondroprotective effect that can be readily appreciated histologically by OARSI scoring in this animal model. The finding that Kellgren/Lawrence scores also showed no significant differences was consistent with these histologic results, although we have observed that multiple lubricin injections in a rat joint with ACLT improves radiographic outcomes (15).

The prevention of the cellular demise of superficial and intermediate zone chondrocytes by lubricin supplementation is a novel finding. It has been noted previously that blunt trauma induces cell death in articular cartilage (32,33), but the effects of other adverse
mechanical stimuli, in this case enhanced friction, were unknown. There is a concern that due to the lack of blunt cartilage trauma, the rat model of ACLT is not a true clinical recapitulation of ACL rupture in mammals. However, other mechanical sequelae of the ACL-deficient joint are resident in this model, such as cellular demise, which appears to be linked to enhanced friction. In related in vitro work in bovine osteochondral plugs, we observed the prevention of apoptosis of superficial zone chondrocytes through the addition of human synoviocyte lubricin (34).

Chondroprotection following lubricin supplementation may have supported more normal weight bearing in the affected rat limb. Our analysis of the rat gait was limited to an analysis of the maximum force differential between the right and left hind limbs. We observed that lubricin-treated rats consistently demonstrated a greater ratio of maximum force of the left hind limb to maximum force of the right hind limb as compared to PBS-treated or untreated rats with ACLT. The ratio of maximum force of the left hind limb to maximum force of the right hind limb was almost significantly lower in lubricin-treated animals than in the control sham-operated animals. The lubricin-treated rat knee with ACLT may have supported more force relative to its contralateral counterpart, which may be due to an inflammatory response from a unilateral joint injury with systemic manifestations, affecting the contralateral synovium. Gait analysis in rodents receiving monarticular interleukin-1β also showed a subtle, but reproducibly quantifiable, gait disturbance, indicating disease progression (35). In future studies, we plan to prospectively monitor changes in gait as animals become lame and are then treated with lubricin. It is possible that the rats with ACLT may have transferred more weight bearing to the front paws, but our analysis was limited to the maximum force differential between the right and left hind limbs.

The observation that urinary CTX-II, a validated marker of type II collagen degradation (36), normalized to creatinine was less concentrated in the lubricin-treated animals is an important translational finding. Twenty-four–hour urine collection from each animal at 2 different time points showed a significant increase in urinary CTX-II in the untreated rats with ACLT. On day 35, PBS-treated animals had a higher urinary CTX-II level than untreated animals with ACLT. Treatment with human synoviocyte lubricin led to statistically significant reductions in urinary CTX-II level compared to no treatment and PBS treatment. Human synoviocyte lubricin–treated rats demonstrated similar levels of urinary CTX-II on day 35 and day 70 after surgery. The magnitude of urinary CTX-II level reduction in the animals in the present study was similar to the reduction seen in a study using twice weekly, albeit less concentrated, injections of human synoviocyte lubricin for 3 weeks (11). Although urinary CTX-II levels in animals treated with human synoviocyte lubricin remained significantly higher than those in capsulotomized control animals on day 70, the single intraarticular injection of human synoviocyte lubricin prevented an increase in urinary CTX-II from day 35 to day 70.

Despite the fact that urinary CTX-II is enzymatically generated, the addition of an antiadhesive, such as lubricin, may prevent the interaction of inflammatory cells and other proteins in SF with the articular surface, in addition to providing boundary lubrication. In the absence of lubricin, biofouling of the articular cartilage with globular proteins is suspected to occur in the lubricin-null mouse (29). In inflammatory arthropathies, lubricin may reduce initial type II collagen cleavage by minimizing superficial zone degradation. Preserving type II collagen architecture in articular cartilage is a critical step in ultimately restoring cartilage after a traumatic injury (37). Loss of GAG precedes catabolism of type II collagen. The significant differences in urinary CTX-II in lubricin-treated versus non–lubricin-treated groups suggests that exogenous lubricin could play a critical role in a posttraumatic strategy to prevent cartilage degeneration.

Lubricin interacts with collagen (38), hydrophobic (39), and hydrophilic surfaces via its C-terminus (40), and likely forms a loop in its mucin domain, allowing the N-terminus to also interact with the same surface (39,41). Surface-bound layers of lubricin appear as both single-end grafted and double-end grafted molecules (42). The O-linked glycosylations which decorate the mucin domain are important in helping to create steric repulsion and hydration shells which manifest as repulsive forces (43) working in an antiadhesive capacity and keep cartilage asperities from direct contact. Lateral translation of one bearing against its apposed bearing is thus “lubricated” under the conditions that characterize mammalian diarthrodial joints, including very slow sliding speed, high pressures, and compliant bearing materials (cartilage) (44).

Boundary lubrication becomes the predominant mode of joint lubrication once loaded and pressurized cartilage has stayed in position for up to 10 minutes and the coefficient of friction has reached an asymptotic level of 0.14 (45,46). At this point, cartilage has reached zero pore pressure by exuding extracellular matrix fluid. Preventing asperity contact, adhesions, and resultant
damage is provided by this “carpet” (42) of end-grafted lubricin molecules. This system is critical to chondro-protection and can be re-introduced to weight-bearing diarthrodial joints because lubricin is surface active and rapidly adsorbs on surfaces.

Reduced cartilage damage, enhanced chondro-protection, and a decrease in inflammation may lead to a mitigation of subsequent OA progression. The chondroprotected joint may thus benefit on a longer-term basis from enhanced weight bearing since compressive load and shear together up-regulate lubricin expression (47). Recent work on possible mechanisms linking abnormal mechanical stress to chondrocyte degeneration and subsequent OA has focused on the immunostimulatory properties of cartilage breakdown products and subsequent ongoing synovial inflammation as contributors to later OA progression (48). Synovial inflammation has been linked to reduced lubricating capacity (49), more rapid OA progression (50), and increased OA pain symptoms.

As discussed by Scanzello et al in their review comparing OA progression to a chronic wound (48), it is particularly worth noting that synovitis is prominent in OA, and that the early inflammatory responses common in both infection and tissue damage occur via the activation of Toll-like receptors. These receptors are activated not only by pathogen surface molecules as occurs in infection, but also by reduced molecular weight hyaluronic acid and fibronectin, which may be released into the joint following injury (48,51). Alternatively, the novel observation that apoptosis is present and responsible for cell death in the joint with ACLT, and not present in the lubricin-injected joint, suggests that the loss of lubricating ability may possibly initiate apoptosis in and of itself (34). Reintroducing lubricin could potentially preserve chondrocytes and the tissue matrix through its regeneration.

In conclusion, this study demonstrates that lubricin is a disease-modifying protein that may prevent the fundamental processes which can lead to postransfermic OA following ACL injury. A single concentrated injection of lubricin appears to be generally equivalent to multiple injections (11) with the same final mass, but further studies of differing dosing strategies are needed. The strategy of once-weekly injections for 3 weeks should be explored. Lubricin is a promising biologic candidate since it is a replacement for a normally occurring mucinous glycoprotein and thus has a low toxicity profile. Patients seek medical attention on average 6.7 days following an acute joint injury (49), which was part of the justification for delayed treatment in this study. Injecting lubricin (tribosupplementation) into the intraarticular space would be an adjunct to viscosupplementation. Human recombinant lubricin will likely have chondroprotective effects similar to those of the synovocyte lubricin used in this investigation (11).

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Jay had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study conception and design. Jay, Elsaid, Teeple, Fleming. Acquisition of data. Jay, Elsaid, Kelly, Anderson, Zhang, Teeple, Waller. Analysis and interpretation of data. Jay, Elsaid, Zhang, Teeple, Waller, Fleming.

ADDITIONAL DISCLOSURES

Authors Kelly and Anderson are employees of Biomodels.

REFERENCES