Osteoarthritis and Cartilage 20 (2012) 940–948

Osteoarthritis and Cartilage

The impact of forced joint exercise on lubricin biosynthesis from articular cartilage following ACL transection and intra-articular lubricin's effect in exercised joints following ACL transection

K.A. Elsaid †*, L. Zhang ‡, K. Waller §, J. Tofte ‡, E. Teeple ||, B.C. Fleming §||, G.D. Jay ‡§

† Department of Pharmaceutical Sciences, Massachusetts College of Pharmacy and Health Sciences, Boston, MA 02115, USA
‡ Emergency Medicine, Rhode Island Hospital, Providence, RI 02903, USA
§ Department of Engineering, Brown University, Providence, RI 02903, USA
|| Department of Orthopaedics, Rhode Island Hospital, Providence, RI 02903, USA

ARTICLE INFO

Article history:
Received 27 July 2011
Accepted 28 April 2012

Keywords:
Lubricin
Cartilage degeneration
ACL
Forced exercise

SUMMARY

Objective: To evaluate the impact of forced joint exercise following acute knee injury on lubricin metabolism and its relationship to cartilage degeneration and to assess chondroprotection of a single-dose purified human lubricin injection in exercised injured joints.
Methods: Anterior cruciate ligament transection (ACLT) was performed in rats with six experimental groups; 3-week post-ACLT, 3-week post-ACLT + exercise, 5-week post-ACLT, 5-week post-ACLT + exercise, and 5-week post-ACLT + exercise treated with intra-articular phosphate buffered saline (PBS) or lubricin. Joint exercise was achieved using a rotating cylinder at a speed of 6 rpm for 30 min daily, 5 days a week starting 1 week following surgery. Cartilage lubricin expression in injured joints was determined. Histological analyses included Safranin O/Fast Green, activated caspase-3, and lubricin mRNA *in-situ* hybridization. Assessment of cartilage damage was performed by osteoarthritis research society international (OARSI) modified Mankin scoring and urinary CTXII (uCTXII) levels.
Results: At 3 weeks, lubricin expression in exercised ACLT joints was significantly ($P < 0.001$) lower compared to ACLT joints. The OARSI scores were significantly ($P < 0.001$) higher in the ACLT + exercise animals compared to ACLT animals at 5 weeks. Compared to 3-week ACLT, 3-week ACLT + exercise cartilage showed increased caspase-3 staining. Compared to ACLT + exercise and PBS-treated ACLT + exercise, lubricin intra-articular treatment resulted in a significant increase ($P < 0.001$) in cartilage lubricin gene expression and a reduction ($P < 0.05$) in uCTXII levels.
Conclusion: Joint exercise resulted in decreased lubricin cartilage expression, increased cartilage degeneration and reduced superficial zone chondrocyte viability in the ACLT joint. Intra-articular lubricin administration ameliorated cartilage damage due to exercise and preserved superficial zone chondrocytes' viability.

Introduction

Acute knee injuries involving cruciate ligament tears, is a significant risk factor for development of post-traumatic osteoarthritis (OA)[1–3]. The mechanism of cartilage degeneration following an acute injury is multifaceted and may involve joint instability, inflammation and possibly loss of joint lubrication[4,5]. Joint lubrication is provided in part by lubricin/proteoglycan 4 (PRG4), a glycoprotein secreted from synoviocytes[6] and superficial zone chondrocytes[7]. In addition to its boundary lubricating properties[8,9], lubricin prevents synoviocyte overgrowth, protects cartilage surfaces and prevents cartilage wear[10,11].

Patients with anterior cruciate ligament (ACL) injuries are typically prescribed an exercise regimen as part of their rehabilitation process. However, the impact of joint exercise following ACL injury on long-term cartilage integrity, superficial chondrocyte viability and joint lubrication mediated by lubricin is not understood. In this investigation, we utilized the rat ACL injury model[12–14] to evaluate the impact of an exercise regimen on lubricin cartilage biosynthesis and its relationship to superficial zone

* Address correspondence and reprint requests to: K.A. Elsaid, Department of Pharmaceutical Sciences, Massachusetts College of Pharmacy and Health Sciences, Boston, MA 02115, USA. Tel: 1-401-575-6137.
E-mail address: khaled.elsaid@mcphs.edu (K.A. Elsaid).

1063-4584/$ – see front matter
doi:10.1016/j.joca.2012.04.021

chondrocytes' viability and cartilage degeneration. We hypothesized that joint exercise following injury will modulate lubricin metabolism leading to accelerating cartilage degeneration. We also posit that lubricin expression by chondrocytes will be enhanced through the intra-articular lubricin supplementation which will serve to prevent cartilage degeneration compared to non-lubricin-treated counterparts.

Methods

ACL transection (ACLT) in the rat

ACLT was performed in 8–10 weeks old male Lewis rats ($n = 144$) which were randomly assigned to six experimental groups; (1) 3-week post-ACLT ($n = 24$), (2) 3-week post-ACLT + exercise ($n = 24$), (3) 5-week post-ACLT ($n = 24$), (4) 5-week post-ACLT + exercise group ($n = 24$), (5) 5-week post-ACLT + exercise treated with one intra-articular sham injection of phosphate buffered saline (PBS) ($n = 24$) and (6) 5-week post-ACLT + exercise treated with one intra-articular injection of purified human lubricin ($n = 24$). Following anesthesia with intraperitoneal Ketamine and Dexmedetomidine, the right knee joint skin was shaved, cleaned with a topical antiseptic, and a lateral skin incision was performed to gain access to the joint capsule. After the joint capsule was opened, the ACL was severed using a surgical scalpel. A positive anterior draw test confirmed the transection of the ACL. Closure of the joint capsule was performed with biodegradable sutures and the animal skin was finally closed using surgical staples. In all animals, the right knee joint was the ACLT joint. All surgeries were performed by KE and all required approvals were obtained from MCPHS IACUC committee prior to the commencement of the study. Control animals ($n = 5$) were age and sex-matched to the operated animals and were used for histological comparisons.

Forced joint exercise

Consistent doses of joint exercise were achieved in the animals assigned to the exercise groups by placing each rat on a custom rotating cylinder apparatus that rotated toward the animal, at a speed of 6 rpm for 30 min daily, 5 days a week starting 1 week following surgery. Animals were trained for 1 week on the rotating cylinder prior to ACLT. This exercise regimen was used to simulate repeated joint flexion and extension.

Intra-articular lubricin and PBS (sham) injections

One week following ACLT, animals were anesthetized with isoflurane and received intra-articular injections of purified human lubricin, obtained from patients undergoing knee and hip joint replacement as previously described[6], at a dose of 40 μg in 50 μl or PBS. Animals were injected through the patellar tendon of the operated knee joint.

Quantitative lubricin expression

Immediately following joint harvest, tibial plateau cartilage was carefully extracted from operated and contralateral joints of ACLT animals at 3 and 5 weeks following surgery, with or without exercise and from animals that received PBS or lubricin ($n = 9$ in each group). Tibial plateau cartilage was immediately snap-frozen and stored at −80°C until RNA isolation. Tibial cartilage tissue was pulverized using a mortar and pestle. Total RNA was isolated from synovial tissue, using a Ribopure kit (Ambion, Austin, TX, USA). Contaminating genomic DNA was removed by DNase I treatment of isolated RNA. Messenger RNA was converted to cDNA using a Cells-to-cDNA II kit (Ambion). Quantitative polymerase chain reaction (PCR) assays were performed as previously described[15] using lubricin 5′-GGAACCGATCTCTTGGTTGA-3′ (forward), 5′-ATCCACTGGCTTACCATTGC-3′ (reverse) and GAPDH 5′-CAGTGCCAGCCTCGTCTCAT-3′ (forward) and 5′-AGGGGCCATC CACAGTCTTC-3′ (reverse) primers.

In-situ lubricin hybridization

Representative articular cartilage specimens ($n = 9$ in each group) were sectioned and probed using a 373-bp fragment corresponding to nucleotides 1283–1655 of the rat PRG4 NCBI RGD: 1308976 gene. Random-primed digoxigenin (DIG)-labeled probe was used and the hybridized probes were immunodetected with anti-DIG-fluorescein Fab fragments (Roche Applied Science) as previously described[16–18]. Fluorescence microscopy was utilized to image the cartilage specimens using a Nikon E800 microscope using a 40× Plan Fluor objective and a Spot II digital camera (Diagnostic Instruments). All images were acquired with the same camera settings. Image processing and analysis were performed using iVision (Biovision, Exton, PA, USA) image analysis software. Positive staining was defined through intensity thresholding and integrated optical density (IOD) was calculated by examining the thresholded area multiplied by the mean[13].

Lubricin concentrations in synovial fluid (SF) lavages

Lavaging of SF from ACLT joints at 3 or 5 weeks ACLT with or without exercise as well as 5-week ACLT joints that received intra-articular PBS or lubricin injections was performed by injecting 100 μl of normal saline in the joint capsule followed by flexing and extending the articular joint for 10 times. A total of 30 μl of fluid was aspirated. Lubricin SF lavage concentrations were determined using a sandwich enzyme-linked immunosorbent assay (ELISA) using peanut agglutinin (PNA; Sigma–Aldrich) and monoclonal antibody 9G3 as previously described[12].

Histological analysis and immunostaining

Paraffin-embedded coronal sections were taken from weight-bearing areas of the articular cartilage of ACL transected joints of each animal ($n = 6$ in each group). Microtomed sections were collected every 250 μm to find a representative area showing both femoral condyles, tibial plateaus, and the menisci. Adjacent sections were collected through this region and stained with Safranin O/Fast green for histological scoring and assessment of sulfated glycosaminoglycans (sGAG) content, immunoprobed with antibody 9G3, a monoclonal antibody specific for lubricin (provided by M. Warman) to evaluate the presence of lubricin, and rabbit polyclonal antibody against active caspase-3 (Abcam, Cambridge, MA, USA) to assess chondrocyte apoptosis. mab 9G3 was incubated with the histological specimens at 1:200 dilutions followed by biotinylated anti-mouse IgG at 1:500 dilution and detected using the Vectastain ABC kit (VECTOR Laboratories, Burlingame, CA, USA). Activated caspase-3 antibody was incubated with the histological specimens at 1:50 dilution followed by biotinylated anti-rabbit IgG at 1:200 dilution and detected using the Vectastain ABC kit.

Histological scoring

The osteoarthritis research society international (OARSI) modified Mankin scoring was utilized to assess cartilage degeneration in the different groups[19]. We did not examine osteophyte formation as part of reported scores. Scoring was performed on the medial and