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Lubricin/proteoglycan 4 increases in both experimental and naturally occurring equine osteoarthritis

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SUMMARY

Objective: The goals of this study were (1) to quantify proteoglycan 4 (*PRG4*) gene expression; (2) to assess lubricin immunostaining; and (3) to measure synovial fluid lubricin concentrations in clinical and experimental models of equine carpal osteoarthritis (OA).

Design: Lubricin synovial fluid concentrations and cartilage and synovial membrane *PRG4* expression were analyzed in research horses undergoing experimental OA induction ($n = 8$) and in equine clinical patients with carpal OA ($n = 58$). Lubricin concentrations were measured using a custom sandwich enzyme-linked immunosorbent assay, and *PRG4* expression was quantified using qRT-PCR. Lubricin immunostaining was assessed in synovial membrane and osteochondral sections in the experimental model.

Results: Lubricin concentrations increased in synovial fluid following induction of OA, peaking at 21 days post-operatively in OA joints vs sham-operated controls (331 ± 69 μg/mL vs 110 ± 19 μg/mL, $P = 0.001$). Lubricin concentrations also increased in horses with naturally occurring OA as compared to control joints (152 ± 32 μg/mL vs 68 ± 4 μg/mL, $P = 0.003$). Synovial membrane *PRG4* expression increased nearly 2-fold in naturally occurring OA ($P = 0.003$), whereas cartilage *PRG4* expression decreased 2.5-fold ($P = 0.025$). Lubricin immunostaining was more pronounced in synovial membrane from OA joints as compared to controls, with intense lubricin localization to sites of cartilage damage.

Conclusions: Although *PRG4* gene expression decreases in OA cartilage, synovial membrane *PRG4* expression, synovial fluid lubricin concentrations and lubricin immunostaining all increase in an equine OA model. Lubricin may be elevated to protect joints from post-traumatic OA.

Introduction

Lubricin, a mucinous glycoprotein encoded by the proteoglycan 4 (*PRG4*) gene, functions as both a boundary lubricant and chondroprotective agent in synovial joints. Patients with camptodactyly-arthropathy-coxa vara-pericarditis (CACP) fail to express *PRG4* and subsequently develop early-onset polyarthropathy[1,2]. Studies have revealed that lubricin-deficient synovial fluid in a subset of patients with osteoarthritis (OA) fails to lubricate cartilage and that boundary lubrication can be restored by the addition of exogenous recombinant lubricin[3]. In human patients with anterior cruciate ligament (ACL) injury[4] and in rodent models of ACL injury[4–6], synovial fluid lubricin concentrations are decreased in injured joints as compared to controls. Synovial fluid lubricin concentrations were also decreased in a population of human patients with late-stage OA and RA[7]. Mounting evidence suggests that lubricin supplementation, either through genetic overexpression[8] or recombinant lubricin supplementation[9–12] delays the progression of OA in rodent models. Accordingly, there is considerable interest in the use of lubricin supplementation as a potential therapeutic for OA, but information about lubricin expression and tissue localization in large animal naturally occurring OA and translational large animal experimental models of OA is limited.

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Large animal models such as the horse more closely recapitulate OA in humans than small animal models because cartilage thickness and joint volume more closely approximate human cartilage[13–15], and cartilage is subject to loading forces of similar or greater magnitude than human cartilage[15]. Moreover, the equine carpal fragment model has the added benefits of allowing repeated synovial fluid sampling and controlled, athletic exercise[15,16]. However, there is substantial controversy about how lubricin is altered in large animal models of arthritis. In one report, *PRG4* mRNA levels were significantly decreased in a sheep meniscectomy model 3 months post-operatively, and gene expression correlated with decreased lubricin immunostaining in degenerative articular cartilage[17]. Conversely, lubricin synovial fluid concentrations and boundary lubrication properties were similar between operated and contralateral control limbs, leading investigators to conclude that lubricin should be evaluated at earlier time points in the development of OA[18]. In dogs undergoing unilateral cranial cruciate ligament transection, lubricin immunohistochemical staining did not differ between OA and control limbs 13 weeks post-operatively[19], and quantitation of lubricin using Western blot analysis revealed an 83% increase in lubricin in acutely injured ($\leq$3 weeks) equine joints[20]. Thus, although several studies in rodent models suggest that lubricin decreases in experimental arthritis, it remains unclear whether lubricin levels are increased, decreased or unchanged in large animal models of OA and over what time course these changes occur.

Furthermore, some investigations report increased synovial fluid lubricin concentrations in human patients with intra-articular fracture[21] or late-stage OA[22], bringing into question whether it is appropriate to extrapolate from rodent models to humans. A primary limitation of studies to date evaluating lubricin synovial fluid concentrations in human patients[3,5] and in most experimental animal models of OA is that synovial fluid lubricin concentrations are only evaluated at a single time point[6,18,20]. A recent longitudinal analysis of synovial fluid lubricin concentrations in an ovine anterior cruciate ligament transection model revealed increased lubricin concentrations at 2 and 4 weeks post-injury as compared to 20 weeks; however, synovial fluid samples were not evaluated prior to injury[23]. Because serial lubricin quantitation has been limited, it is difficult to make inferences as to how lubricin concentrations change over the course of OA, whether altered lubricin concentrations precede the development of OA, and whether or not intra-articular lubricin supplementation may be indicated in clinically relevant large animal models or humans. To our knowledge, no studies have evaluated *PRG4* expression, serial synovial fluid lubricin concentrations and immunostaining in the same model.

Our objective, therefore, was to assess changes in synovial fluid lubricin concentrations at serial intervals both before and after osteochondral fragmentation in an equine OA model[16], and to assess *PRG4* expression and immunostaining from articular cartilage and synovium at study termination 70 days post-OA induction. In addition, we sought to quantify *PRG4* and lubricin glycoprotein expression in cartilage, synovial membrane and synovial fluid samples from horses with naturally occurring carpal OA injuries similar to the carpal osteochondral fragment experimental model. We hypothesized that *PRG4* expression, lubricin synovial fluid concentrations and lubricin synovial membrane and cartilage immunostaining would decrease in both experimental and naturally occurring OA in horses.

Methods

Samples

PRG4 expression and lubricin synovial fluid concentrations were analyzed in samples from two equine cohorts: equine clinical patients or research animals with carpal OA ($n = 36$) or healthy carpal joints ($n = 22$), and research horses undergoing carpal osteochondral fragmentation for OA induction ($n = 8$). All experimental protocols were approved by the Cornell University Institutional Animal Care and Use Committee, and all synovial fluid and tissue samples were collected with informed owner consent.

Naturally occurring OA

Healthy and OA synovial fluid, synovial membrane and cartilage tissues were harvested from the antebrachiocarpal (ACJ) and middle carpal (MCJ) joints of horses presenting to the Cornell University Equine Hospital for arthroscopy or from horses donated to the hospital for research purposes. Cytokine and catabolic enzyme expression from this cohort of primarily Thoroughbred horses ranging in age from 2 to 11 years has been previously described[24]. Radiographic evaluation of all OA joints was performed prior to surgery or euthanasia, and joints were assigned a score of normal, mild, moderate or severe OA according to the radiographic presence of osteophytes, enthesiophytes, osteoproliferation, joint space narrowing, or chronic fracture lines, with additional arthroscopic/gross scoring used to corroborate the radiographic score. Thirty-six horses with carpal OA underwent radiography prior to surgery or euthanasia, and 22 horses with normal joints were included in the study.

Experimental OA

The arthroscopically created equine carpal osteochondral fragment-exercise model of OA has been previously described[16,25]. Briefly, an 8 mm osteochondral fragment was created at the distal dorsal aspect of the radial carpal bone of one randomly assigned forelimb. The contralateral forelimb was sham-operated without creation of a fragment. Synovial fluid samples were obtained from both limbs at the time of initial arthroscopy and at weekly intervals post-operatively. Horses were maintained in 3.65 m $\times$ 3.65 m box stalls and were exercised on a high-speed treadmill 5 times weekly beginning 2 weeks post-operatively and continuing for the study duration to simulate race training. Horses were walked (5 km/h) for 4 min, trotted (16–19 km/h) for 2 min, galloped (28–32 km/h) for 2 min, and trotted (16–19 km/h) for 2 min, for a total exercise time of 10 min. On day 70 post-induction, horses were euthanized with an overdose of barbiturate, and synovial membrane biopsies obtained for RNA expression. Synovial fluid was collected, and synovial membrane and osteochondral blocks, including the radial carpal bone fragment and parent bone and the opposite third carpal bone, were harvested for histological processing and immunohistochemistry. Eight Thoroughbred horses ($n = 5$ females and $n = 3$ castrated males), aged 2–6 years old, were used for the study.

Isolation of RNA and real-time quantitative PCR lubricin assay

Synovial membrane tissue and cartilage was snap-frozen in liquid nitrogen, pulverized and stored at −80°C prior to isolation of RNA using the PerfectPure RNA Tissue Kit (5Prime, Gaithersburg, MD), and RNA purity and concentration were assessed with UV microspectrophotometry (NanoDrop 2000 Spectrophotometer, Thermo Scientific, Waltham, MA). *PRG4* gene expression was quantified by real-time PCR using the Taqman One-Step RT-PCR technique (Absolute Quantitative PCR; ABI PRISM 7900 HT Sequence Detection System, Applied Biosystems, Foster City, CA). Primer Express Software Version 2.0 (Applied Biosystems, Foster City, CA) was used to design primers and dual-labeled fluorescent probes (6-carboxyfluorescein (FAM) as the 5′ reporter dye and Iowa Black® FQ as the 3′ quenching dye) for quantification of equine *PRG4*. Primers were designed as follows: Fwd: 5′ – TGCGGTGCTTCCCCATAC – 3′; Rev: 5′ – AAACAGGAACCCATCA