Lubricin Increases in Osteochondral Fragment-induced and Naturally Occurring Osteoarthritis in Horses

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Disclosures:

Introduction: Lubricin, a mucinous glycoprotein encoded by the proteoglycan 4 (PRG4) gene, functions as both a boundary lubricant and chondroprotective agent in the synovial environment. Mounting evidence suggests that lubricin delays the progression of osteoarthritis (OA)[1,2], and human patients with anterior cruciate ligament injury[3] as well as rodent models of post-traumatic osteoarthritis[4] demonstrate decreased synovial lubricin concentrations as compared to normal joints. Accordingly, there is considerable interest in the use of lubricin supplementation as a potential therapeutic for OA in human clinical patients. However, data supporting the use of lubricin in a clinically relevant, translational animal model is lacking. Moreover, Antonacci et al. recently demonstrated increased lubricin in synovial fluid samples from equine patients with early OA undergoing carpal and fetlock arthroscopy. Our objective, therefore, was to assess changes in PRG4 gene expression and lubricin synovial fluid concentrations at weekly intervals after osteochondral fragmentation in a well-validated OA model in horses[5] as well as in naturally occurring equine OA. We hypothesized that PRG4 gene expression and lubricin synovial fluid concentrations would decrease in both the carpal osteochondral fragment model of OA and naturally occurring OA in horses.

Methods: Lubricin synovial fluid concentrations and PRG4 gene expression were analyzed in two separate equine populations: research horses undergoing osteochondral fragmentation for OA induction, and equine clinical patients or research donations (n=58). All studies were conducted under IACUC approval.

Osteochondral fragment model: An 8mm osteochondral fragment was created in the middle carpal joint of one randomly assigned limb of 8 Thoroughbred horses, aged 2-6 years old. The contralateral forelimb was a sham-operated control. Synovial fluid samples were obtained from both limbs at the time of initial arthroscopy and at weekly intervals post-operatively. Horses were exercised on a high-speed treadmill 5 times weekly for the study duration. On day 70 post-induction, horses were euthanized and synovial membrane biopsies obtained for RNA expression.

Naturally occurring OA: Thirty-six horses with carpal OA underwent radiography prior to surgery or euthanasia and were scored as mild (1), moderate (2), or severe (3) OA according to radiographic analysis of osteophytes, enthesiophytes, osteoproliferation, joint space narrowing, or chronic fracture lines. Twenty-two horses with normal joints were included in the study. Horses were primarily Thoroughbred, ranging in age from 2 to 11 years.

RNA was extracted from synovial membrane and cartilage, and gene expression quantified using primers and probes generated from equine C-terminus PRG4 clones. Copy number was quantified for PRG4 mRNA with 18S used as a housekeeping gene. Data used for statistical analysis was copy number per ng. Synovial fluid lubricin was quantified using a peanut agglutinin sandwich ELISA with mAb 9G3 against the mucin domain of lubricin (courtesy G Jay). Purified equine lubricin was used as a standard. All data are presented as means ± standard error (s.e.). The differences in mean [lubricin] between OA and control limbs in the osteochondral fragment model were fit to a linear regression model with time and time^2 as covariates. Means for OA vs. control joints were compared using a 2-sample t-test. Synovial fluid and qRT-PCR data were analyzed using a 1-way ANOVA with Tukey’s HSD post hoc tests. Comparisons between normal and OA joints (1, 2, and 3 grouped together) were made using a 2-sample t-test. Log values were used to normalize cartilage gene expression data, and summary statistics were performed on the untransformed data. A p-value < 0.05 was considered significant.

Results: Osteochondral fragment model: Lubricin concentrations increased in synovial fluid following induction of OA (p=0.0003), peaking at 21 days post-operatively in joints with carpal fragmentation vs. sham-operated controls (330.9 ± 69.2 ug/mL vs. 110 ug/mL ± 19.4 ug/mL) and remaining elevated over controls throughout day 70 (221 ± 41.7 ug/mL vs. 151.1 ± 20.1 ug/mL) (Fig. 1). There was a significant effect of time (p=0.0300) and time2 (p=0.0201) on lubricin synovial fluid concentrations after fragment induction, with only a transient elevation in lubricin concentrations in sham-operated controls, peaking at seven days post-operatively (Fig. 1). The elevation in lubricin concentrations in synovial fluid did not correspond to an increase in PRG4 synovial membrane gene expression in horses with OA vs. controls (1.85x10^5 ± 0.21x10^5 copies/ng vs. 1.77x10^5 ± 0.19x10^5 copies/ng) at day 70 post-carpal fragment induction.

Naturally occurring OA: Synovial fluid lubricin concentrations in equine clinical cases (n=47) demonstrated a trend for increased levels in all grades of naturally occurring OA as compared to controls (Fig. 3). Because there were no significant differences between OA severity grades, when grouped together (1, 2, and 3), lubricin concentrations were significantly elevated in OA vs. normal joints (151.7 ± 32.2 ug/mL vs. 67.6 ± 4.3 ug/mL, p=0.0157). Synovial membrane PRG4 gene expression increased in
naturally occurring OA; whereas cartilage PRG4 transcription levels decreased (Fig. 4). Differences in PRG4 gene expression were statistically significant between control and moderate OA cases for both synovial membrane (p=0.0025) and cartilage (p=0.0248).

**Discussion:** Contrary to our hypotheses, the results of the present study indicate that synovial fluid lubricin concentrations increase in response to osteochondral fragmentation and naturally occurring OA in horses, and synovial membrane PRG4 transcription increases in naturally occurring OA. Conversely, in naturally occurring OA, cartilage PRG4 gene expression decreases. Synovial fluid lubricin concentrations in clinical cases paralleled the trend for the osteochondral fragment model, with increasing lubricin concentrations associated with OA. Limitations of this study are the lack of information on molecular degradation of lubricin and the qualitative nature of the OA grading criteria for naturally occurring cases combined with minimal information on duration of injury prior to presentation.

**Significance:** Lubricin supplementation has been proposed as a novel OA therapy in humans, with mounting evidence for its clinical application. The results of the present study suggest that lubricin is already increased in synovial fluid following traumatic osteochondral injury in horses. Further investigation is warranted in clinically relevant large animal models prior to translation to humans.

**Acknowledgments:** The assistance of Dr. Hussni Mohammed with statistical analysis is gratefully acknowledged.

**References:**

**Figure 1.** (A) Synovial fluid lubricin concentrations sampled immediately prior to carpal arthroscopy (Day 0) and at weekly intervals after carpal osteochondral fragment induction (OA, n=8) vs. sham-operated controls (Control, n=8). (B) PRG4 transcription levels in synovial membrane from control (n=8) and OA (n=8) joints at day 70 after carpal osteochondral fragment induction. There were no significant differences in PRG4 levels between groups (p<0.05). Data are presented as mean ± s.e.
Figure 2. Synovial fluid lubricin concentrations from naturally occurring OA cases. Categories were numbered as normal (0), mild OA (1), moderate OA (2) and severe OA (3). Increased lubricin concentrations in OA cases were not statistically significant at p<0.05. Data are presented as mean ± s.e.

Figure 3. PRG4 transcription levels in synovial membrane (n=58) and cartilage (n=29) from naturally occurring OA cases. Categories were numbered as normal (0), mild OA (1), moderate OA (2) and severe OA (3). Data are presented as mean ± s.e. Differing letters indicate significant difference at p<0.05.