Lubricin Supplementation Prevents Osteoarthritis Progression in a Rat Surgical Model

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Introduction: The chondroprotective glycoprotein lubricin contributes a vital defense against the pathologic wear and degeneration of articular cartilage [1,2], suggesting that local delivery of recombinant lubricin could offer substantial benefits as a disease-modifying treatment for osteoarthritic joints. We generated a novel lubricin protein construct, “LUB:1”, and evaluated its therapeutic efficacy following intraarticular administration in an experimental (meniscal tear-induced) rat model of osteoarthritis (OA). Supplementation with LUB:1 initiated one week post-surgery, significantly reduced multiple parameters of OA progression over a subsequent 4 week period, supporting the potential use of recombinant lubricin for the treatment of degenerative joint diseases.

Materials and Methods: Production of recombinant lubricin construct LUB:1—

cDNA for human lubricin [3] was subjected to restriction enzyme digestion at unique sites in exon 6 which flank the sequence encoding Ala374-Pro847 (474 amino acids). This region was replaced with a short, synthetic cDNA cassette encoding 7 ‘KEPAPTT-like’ repeat elements [4]. The resulting construct, designated “LUB:1”, maintains the native human lubricin exons 1-5 (encoding the signal peptide and N-terminal domains), 5’ and 3’ regions of exon 6, and exons 7-12 (encoding C-terminal domains). LUB:1 was expressed in a stably-transfected CHO cell line, and purified from serum-free conditioned media.

Evaluation of LUB:1 efficacy in a rat model of OA—
The basic study design and animal usage was IACUC approved. Unilateral OA was induced in the knees of male Lewis rats (n=20 animals/treatment group) by transection of the medial collateral ligament and meniscus (meniscal tear model). Beginning one week post-surgery, LUB:1 (20μg/40μl) or phosphate-buffered saline (PBS) vehicle (40μl) was administered by intraarticular injection, with dosing three times per week over a four week period. At the end of the study, tissue sections of each injected knee joint were analyzed microscopically and assessed for multiple pathological parameters of OA [5]. Statistical analysis was performed using a Student’s two-tailed t-test, with significance set at p≤0.05.

Results: A recombinant lubricin protein construct, LUB:1, was efficiently expressed in CHO cells, and purified from CHO culture media. LUB:1 was shown to be functionally active in vitro, as demonstrated by its ability to prevent cell adhesion and growth on coated tissue culture wells [1], in contrast to equimolar amounts of a control protein (bovine serum albumin).

In a rat meniscal tear model of OA, tibial cartilage degeneration scores were significantly reduced by 22% (p=0.022) for LUB:1-treated versus PBS-treated animals (Fig. 1A). Osteophyte size and total joint scores were also significantly lowered by 14% (p=0.017) and 15% (p=0.016), respectively, following LUB:1 supplementation. Strikingly, marked/severe collagen damage across the medial tibial plateau, reflecting areas of cartilage degeneration in which collagen loss extends through greater than 50% of the cartilage depth, was significantly decreased by 83% (p=0.020) in response to LUB:1 (Fig. 1B). For this parameter, the frequency of such lesions was 10/20 (50%) for PBS-treated animals, and 5/20 (25%) for animals dosed with LUB:1. Subchondral bone changes did not differ significantly between treatment groups, indicating that joint loading was comparable in all animals. In addition, medial capsule width (indicative of synovial fibroplasia) was not significantly different between experimental cohorts.

Discussion: In the current study, we tested the effects of intraarticular lubricin supplementation on disease progression in a rat model of OA. Lubricin is a complex glycoprotein, containing an extensive mucin-like domain (encoded by exon 6) with multiple repetitive ‘KEPAPTT-like’ peptide sequence elements bearing attachment sites for lubricating O-linked oligosaccharides [6,7]. We optimized efficient expression of recombinant human lubricin by replacing most of these repeat sequences with a synthetic ‘cassette’ to generate a novel lubricin protein construct, “LUB:1”. Relative to full-length human lubricin, the LUB:1 sequence contains approximately one-third of the number of ‘KEPAPTT-like’ elements located within the mucin-like domain.

Local administration of LUB:1 was therapeutically effective in preventing cartilage degeneration in a rat model of surgically-induced OA (meniscal-tear model). Thus, the knees from animals treated with LUB:1 developed significantly less severe lesions than PBS (vehicle)-treated animals. The reduction in cartilage degeneration scores (general pathology) and inhibition of loss of viable matrix demonstrated a beneficial effect of LUB:1 on overall cartilage structural preservation.

In addition to further evaluating its utility to treat OA, other potential therapeutic uses for lubricin supplementation may also be envisaged, such as the alleviation of deleterious adhesion formation associated with tendon injury/repair. Furthermore, the synthetic strategy applied for the expression of LUB:1 facilitates iterative expansion of the number of ‘KEPAPTT-like’ sequences within the exon 6-encoded region [4]. Development and characterization of such constructs may serve to further optimize the efficacy of recombinant lubricin molecules.

References: