Early Intra-Articular Interleukin-1 Alpha Receptor Antagonist (IL-1 ra) Administration Increases Lubricin Biosynthesis Following Anterior Cruciate Ligament Injury in the Rat

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Disclosures:

Introduction: Lubricin/PRG4 is a mucinous glycoprotein, secreted by synoviocytes and superficial zone articular chondrocytes that provides boundary lubricating and chondroprotective effects [1, 2]. Following an acute joint injury, lubricin gene expression and synovial fluid (SF) levels are significantly reduced, placing the joint at a risk of cartilage degeneration [3, 4]. Pro-inflammatory cytokines such as interleukin-1 alpha (IL-1α) reduces lubricin gene expression [5]. The objective of this work is to evaluate the impact of antagonizing the effects of IL-1α on lubricin expression, SF lubricin levels and cartilage staining following anterior cruciate ligament transection (ACLT) in the rat.

Methods: ACLT in the rat: ACLT was performed in 9-10 weeks old male Lewis rats (n=72) and were randomly assigned to a PBS or an interleukin-1 receptor antagonist (IL-1 ra) treatment (n=36 in each group). ACLT was performed as described previously [4]. Intra-articular treatments: On days 1, 3, 5 and 7 post-ACLT, animals received 40 µl of sterile PBS or 40 µl of recombinant IL-1 ra (Anakinra; 150 mg/ml). Animals were injected under anesthesia through the patellar tendon of the operated knee joint. At 3 and 5 weeks post-ACLT, animals were sacrificed and their joints were harvested. Quantitative lubricin expression: Immediately following joint harvest, tibial plateau cartilage was carefully dissected from the operated and contra-lateral knees and was immediately snap-frozen and stored at -80°C until RNA isolation (n=9 in each group at each time point). Total RNA isolation followed by quantitative lubricin expression was performed as described previously [4]. Data was expressed as a ratio of lubricin expression in the operated knee to that in the contralateral knee. Lubricin immunostaining: Following joint decalcification, paraffin-embedded coronal sections were taken from the weight-bearing areas of the articular cartilage of the ACLT joints (n=9 in each group at each time point). Micrrotomed sections were collected every 250 µm to find a representative area showing both femoral condyles, tibial plateaus and the menisci. Lubricin immunostaining was performed using lubricin-specific mab 9G3 at 1:200 dilution followed by biotynilated anti-mouse IgG at 1:500 dilution and detected using the Vectastain ABC kit (VECTOR Laboratories, Burlingame, CA, USA). Lubricin quantitation in SF lavages: SF lavaging was performed at 5 weeks post-ACLT in the PBS and IL-1 ra treatment groups by injecting 100 µl of normal saline in the joint capsule followed by flexing and extending the joint for 10 times. A total of 20 µl were aspirated and lubricin SF lavage concentrations were determined using a sandwich ELISA with peanut agglutinin and mab 9G3 as described [4]. Statistical analyses: lubricin expression levels across groups were compared by ANOVA. Lubricin SF lavage concentrations were compared by Student’s t-test with α set at 0.05. Lubricin SF lavage concentrations are reported as mean ± standard deviation.

Results: At 3 weeks post-ACLT, the IL-1 ra-treated animals had significantly higher (p=0.002) lubricin expression compared to PBS-treated animals (Fig. 1). Similarly, lubricin expression level in the IL-1 ra-treated animals at 5 weeks post-ACLT was significantly higher (p=0.001) than in the PBS-treated animals. The lubricin expression in the PBS-treated animals at 5 weeks post-ACLT was significantly lower (p<0.001) than in the PBS-treated animals at 3 weeks post-ACLT. No significant difference in lubricin expression was found between the cartilage from IL-1 ra treated animals at 3 and 5 weeks post-ACLT. Lubricin immunostaining demonstrated intense staining in the superficial zone chondrocytes in control, IL-1 ra treated animals at 3 and 5 weeks post-ACLT (Fig. 2). On the contrary, no chondrocyte staining was observed in the PBS-treated animals at 5 weeks with weak staining at 3 weeks post-ACLT. The mean SF lavage lubricin concentration in the PBS-tREATED group was 63.3±18.7 µg/ml compared to 90.6±26.3 µg/ml in the IL-1 ra treated animals. IL-1 ra treatment has resulted in a significant increase (p=0.022) in mean SF lavage lubricin concentrations compared to PBS treatment.

Discussion: Blocking the effects of IL-1 following an acute joint insult restores lubricin expression in articular cartilage and increases SF lavage lubricin concentrations. This may provide a beneficial effect in reducing cartilage degeneration by restoring lubricin-mediated chondroprotection. Intra-articular delivery of recombinant lubricin itself has a disease-modifying activity in an ACLT rat model [6]. Combining this approach and IL-1 ra may exert a synergistic effect in mitigating cartilage degeneration following acute joint injury.

Significance: Interrupting key pathologic events following an ACL injury can provide disease-modifying effects. Intra-articular administration of an IL-1 receptor antagonist restores boundary lubrication which may be useful in preventing subsequent cartilage degeneration.

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Fig. 2: Representative lubricin immunostained cartilage specimens from non-operated knees (control), PBS-treated ACLT animals at 3 weeks post-ACLT (3 week ACLT-PBS), IL-1 ra treated ACLT animals at 3 weeks post-ACLT (3 week ACLT-IL-1 ra) and the corresponding 5 week animals. Arrows indicate intense lubricin staining in the superficial zone of cartilage with IL-1 ra treatment. Scale represents 50μm.

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