Assessing the Role of Synovial Inflammation, SDF-1, IL-1-β, TNF-α, and Lubricin in the Pathogenesis of Morphologically Similar Natural and Post-traumatic Hartley Guinea Pig OA Models

INTRODUCTION:
Osteoarthritis (OA) is the most common type of joint disease in the world, and is characterized by a progressive biochemical breakdown of articular cartilage. Studies have frequently transected the anterior cruciate ligament (ACL) and aged animals to reproducibly create models for secondary and primary OA, respectively. Although the pathogenesis of each form is largely unknown, it is known that both etiologies have similar morphological characteristics and the destruction of cartilage is accelerated in post-traumatic (secondary) OA compared to idiopathic (primary) OA. Recent research examining unstable joint models has implicated the synovium in increased synthesis of Stromal cell-derived-factor 1 (SDF-1), inducing matrix metalloproteinase (MMP) activity, and release of various cytokines including Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-1 (IL-1β), both of which downregulate lubricin, a chondroprotective protein. However, it is unclear if primary osteoarthritis utilizes a similar pathological mechanism. The first objective of this study was to determine an appropriate time point in which natural and post-traumatic OA in Hartley guinea pigs have similar cartilage damage based on histological analysis. If an appropriate equivalency point was determined, a second objective was to study the extent of synovial hyperplasia and inflammation followed by synovial fluid (SF) analysis of SDF-1, MMP-13, TNF-α, IL-1β, and lubricin.

METHODS:
Animal models: 36 Hartley guinea pigs were obtained, and 3 groups of Hartley guinea pigs (n=6 each) received ACL transection (ACLT) on the right knee at 3 months of age and were sacrificed at 10 weeks (5.5 month-old), 14 weeks (6.5 month-old) and 18 weeks (7.5 month-old) post-surgery. The remaining groups served as the 3 month baseline control, natural OA at 12 months of age, and the sham groups (sacrificed at the same ACLT timepoints). The unoperated, contralateral (CL) sham joints served as a control for the development of primary OA at the ACLT timepoint. Histology: upon sacrifice, Indian Ink staining was used to analyze gross morphology, and Safranin O/Fast green staining assessed cartilage damage quantified by the modified Mankin Score. Scoring was based on structural damage, proteoglycan loss, and cellularity changes. The synovium was evaluated through H&E staining by Pelletier’s grading system, a nine-point system based on synovial lining hyperplasia, villous hyperplasia, and leukocyte infiltration. Three independent, blinded observers recorded the worst scores from different coronal planes and compartments of the joint. SF analysis: SF lavages were obtained after an injection of 100µL normal saline and 10 cycles of flexing and extending the joint. Commercially available sandwich ELISAs quantified the SF concentrations of SDF-1, MMP-13, IL-1β (R&D Systems). Sandwich ELISAs using 9G3, a lubricin-specific monoclonal antibody and peanut agglutin quantified SF lubricin levels (1). Mouse anti-TNFα, polyclonal Rabbit anti-Mouse TNFα biotin conjugate (Invitrogen), and guinea pig TNFα standard (Evotro) were used with BD OptEIA Reagent Set B (BD Biosciences) to quantify TNFα in SF at an absorbance of 450 nm and a background of 570 nm.

RESULTS: 12 month-old guinea pigs had equivalent cartilage damage with that of 5.5 month-old ACLT animals evaluated by the modified Mankin Score (P=.7524). There were no statistically significant differences (P=.166) in cartilage damage in the 5.5 month-old un-operated sham knee compared to the baseline control. Intrarater reliability was excellent (ICC=.948, 95% CI .914 - .970).

DISCUSSION: No studies to date have directly compared idiopathic and post-traumatic models of OA at the same stage of cartilage destruction. 10 weeks-post ACLT of the Hartley guinea pig joint showed equivalent cartilage damage with that of 1-year-old animals, but each possessed a unique cytokine profile. Knee joints of both groups activated SDF-1, MMP-13, and IL-1β at similar levels, suggesting a shared pathway and potential pharmacologic targets for unknown OA etiology. However, ACLT joints possessed more synovitis, explaining its accelerated cartilage damage via upregulation of TNFα, and downregulation of lubricin. This pattern of TNFα and lubricin SF levels may serve as biomarkers for post-traumatic OA in a specific window of time. More importantly, future OA treatment studies may need to differentiate primary and secondary OA in order to tailor therapy.

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