DETECTION OF MENISCAL CHANGES PRIOR TO OVERT DAMAGE FOLLOWING ACL/MCL TRANSECTION IN AN OVINE STIFLE JOINT MODEL OF OSTEOARTHRITIS

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INTRODUCTION: The anterior cruciate ligament (ACL) is one of the most frequently injured ligaments in the knee. ACL injury often leads to significant functional impairment in athletes and is associated with induction of degenerative joint disease. ACL injuries are regarded as “the beginning of the end of the knee”. This is because the incidence of meniscal pathology and chondral defects increases in the chronically unstable knee lacking an ACL. However, the mechanism of meniscal pathology after ACL injury is still unclear.

The purpose of this study was to determine specifically what effects combined ACL/MCL (medial collateral ligament) transection of the ovine stifle joint had on the remaining intact meniscus, especially from a histological, biochemical and molecular perspective. Understanding the bioprocesses of adaptation on these levels could potentially identify biomolecules which might be delivered therapeutically to encourage or reduce the progression of meniscus deterioration.

MATERIALS AND METHODS: Twelve skeletally mature female Suffolk Cross sheep were used in this study which had the approval of our Animal Care Committee. Four animals (eight stifle joints) served as unoperated normal controls while five had a unilateral ACL/MCL transection and three underwent sham operation under general anesthesia. Sheep were walked, incline walked, and trotted on a treadmill daily. At 4-week intervals in vivo kinematic assessments were performed until sacrifice at 20 weeks post surgery [1]. All joints had gross morphological grading [2] followed by harvest of intact menisci. Both medial and lateral menisci were measured and weighed immediately, then dissected into anterior, middle and posterior parts. Each sample was assessed for histological, biochemical and molecular study. Tissues for biochemical analysis were weighed and dried in vacuum to constant weight and then water content calculated. Menisci for histological study were embedded in paraffin, sections cut and stained with Masson’s trichrome and Safranin-O, prior to light microscopic evaluation. The Jackson scales [3] (higher scores indicate worse condition), which is based on cellularity, hyaline cartilage metaplasia, collagen fiber separation, chondrocyte clustering and surface cellularity, was used for histological evaluation. Tissues for RT-PCR were snap frozen in liquid nitrogen and stored at −70 °C until further analysis. RNA was isolated by a method previously described by Reno et al. [4]. Total RNA was quantified fluorometrically using Syby Green II fluorescent RNA dye. Total RNA from all samples was reverse-transcribed using the Qiagen Omniscript RT kit. The PCR was performed with sheep specific primers for type I, II and III collagen, aggrecan, biglycan, decorin, fibromodulin, matrix metalloproteinase-13 (MMP-13), and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1).

Statistical analysis consisted of comparing each meniscus from normal, ACL/MCL transected legs, and sham operated legs using Kruskal-Wallis test and Mann-Whitney’s U test. (p<0.05)

RESULTS: At sacrifice, there was no overt meniscal injury from ACL/MCL transected, sham and normal joints, with the exception of one lateral meniscus in a transected joint which had minimal fibrillation in the posterior horn. Despite no gross morphological changes, water content and histological grading changed site specifically. Both the anterior horn and middle part of medial menisci from ACL/MCL transected joints had significantly higher water content. With histological evaluation, there was more cellularity and hyaline cartilage metaplasia in the samples from ACL/MCL transected joints (Fig. 1). In particular, the posterior horn medial menisci had significantly higher grading scores.

Analysis of mRNA using RT-PCR demonstrated biglycan mRNA levels were significantly lower in the posterior horn of the medial meniscus from ACL/MCL transected joints. All meniscal samples from transected joints had higher MMP-13 levels (3 fold increase). This expression was prominent in the posterior segment of medial and the anterior segment of lateral menisci, where 10 fold increases in MMP-13 mRNA levels were demonstrated. In contrast to MMP-13 mRNA expression, pronounced changes in TIMP-1 mRNA levels were not evidenced (Fig. 2).

DISCUSSION: The results presented in this report demonstrate that combined ACL/MCL transection induced histological, biochemical and molecular site specific changes in menisci.

Previous studies have shown that the posterior horn of the medial meniscus helps to limit the continued anterior translation of the tibia after disruption of the ACL [5]. Alteration in the function of this segment of the meniscus due to ultrastructural changes may lead to impaired function in anterior tibia translational control. This observation is consistent with histological data in our study where the posterior horn of medial meniscus had higher Jackson scale histological grading scores. In the molecular analysis, significant increases in mRNA levels of the matrix degrading enzyme MMP-13 were found in the posterior portion of medial and the anterior portion of lateral menisci. Interestingly, the mRNA expression pattern of both MMP-13 and TIMP-1 showed similarities to MCL scar gene expression, but contrasted those of intact PCL remodeling following ACL/MCL transection. In this study, increased MMP-13 and decreased TIMP-1 mRNA levels were evidenced (data not shown; Combined ORS 2004 presentation). Previous rabbit ACL transection models showed increases in both MMP-13 and TIMP-1 mRNA levels after 3 and 8 weeks with an obvious meniscal tear [6]. Based on this expression pattern, up-regulated levels of matrix degrading enzymes like MMP-13 could lead to meniscal degeneration and altered tissue function.

The velocities of the tibial surface with respect to the femur were significantly altered at 20 weeks post transection in a similar ovine model [1]. Ligament transection caused increases in both the magnitude and direction of the tibial velocities. This increase may induce gene expression. Further analysis to compare degenerative enzyme gene expression and the degree of regional velocity changes will be needed to clarify the relationship between kinematic change and cell reaction in vivo.

While it is still unclear how meniscus degeneration starts and what factors are involved, caution must be taken in interpreting the absence of overt gross morphological changes, since meniscal degeneration may have already started at the histological, biochemical, and molecular level.

REFERENCES:

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