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ABS0281 ACLT+ pMMx SURGERY SUCCESSFULLY INDUCES POST-TRAUMATIC OSTEOARTHRITIS IN SHEEP

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Osteoarthritis (OA) is a chronic degenerative disease that affects the entire joint structure, causing cartilage degradation, abnormal bone remodeling, osteophyte formation and synovial inflammation. Potential disease-modifying OA drugs (DMOAD) need to be evaluated *in vivo* to demonstrate their efficacy at maintaining joint structure and function. The advantage of sheep over other large animal models is the human-like size of the knee joint and the anatomical and biomechanical similarities with the human knee. Histological joint assessment in ovine models is well-described in the literature by the Osteoarthritis Research Society International (OARSI) recommendations [1]. A reproducible, low-invasive, fast-developing and moderately severe animal model of OA is the anterior cruciate ligament transection (ACLT) combined with partial medial meniscectomy (pMMx) model. Although it has been extensively studied in rodents, its application to sheep remains to be explored.

Objectives:

The goal of the present study was to validate the ACLT-pMMx model as a reliable approach to induce OA in sheep by characterizing its effect on cartilage using the OARSI macroscopic and microscopic scores for histological assessments of sheep joints.

Methods:

Thirty-two Ile-De-France female sheep, 2 to 4 years old and weighing 70-90 kg, were subjected to surgical anterior cruciate ligament transection (ACLT) combined with a partial medial meniscectomy (pMMx) of the left knee on day 0 (D0). Twenty-four of these sheep received an intra-articular injection of two different doses of HAPLN1 human recombinant protein (rhHAPLN1) or vehicle only at week 11 and 19. Macroscopical and histological analyses were performed on samples collected 27 weeks (W27) after surgery. Clinical evaluations were performed weekly from the beginning (D0) until the end of the in-life phase (W27). Blood and synovial fluid were collected at D0, as well as after 11, 15, 19, 23, and 27 weeks (W11, W15, W19, W23, and W27). Macroscopic and histological evaluation of the cartilage, synovial membrane and osteophytes was performed according to the OARSI recommendations in sheep [1] by a minimum of two blinded, independent observers. Both the left operated knee and the right contralateral knee were evaluated. In total, 12 zones of cartilage samples were collected: Cranial, central and caudal region each of medial femoral condyle; lateral femoral condyle; lateral tibial condyle; and medial tibial condyle. Total protein content and Coll2-1 biomarker in synovial fluid were assessed according to standard operating procedures.

Results:

The synovial membrane showed significant signs of spread synovitis in the operated knee when compared to the contralateral knee, according to both OARSI macroscopic and microscopic scorings, a well-known consequence of the OA-inducing surgical model. This was confirmed by the significantly higher synovial fluid score found 11 weeks post-surgery as well as by the increased total protein content in the synovial fluid of the operated knees compared to their contralateral counterparts up to 19 weeks post-surgery. Moreover, macroscopic analyses evidenced clear damage of the cartilage, with the medial tibial condyle being the most impacted

followed by the medial femoral condyle, these two regions showing higher OARSI cartilage macroscopic sub-scores. Major structural changes were also observed with an elevated osteophyte OARSI macroscopic sub-score, mainly in the medial tibial condyle, in the left operated knees in comparison to the contralateral knees. Interestingly, the groups treated twice with 10 µg/knee or 100 µg/knee of rhHAPLN1 had significantly lower macroscopic scores in terms of cartilage, osteophyte, and synovial membrane outcomes compared to the vehicle control. Microscopic cartilage alterations were mainly visible on the site of meniscus removal, as evidenced by a significantly increased OARSI microscopic score for the medial tibial condyle in the operated knee compared to the contralateral knee, specifically in the cranial and caudal regions. OARSI microscopic sub-scores also revealed significant structural alterations of the cartilage of the medial tibial condyle in the operated knee: increased erosion and fibrillation of the cartilage layer, lower chondrocyte density, and abnormal chondrocyte cell cloning, while interterritorial staining and tidemark remained unaltered. Interestingly, exacerbated cartilage degradation in the operated knee was confirmed by elevated levels of Coll2-1 in the synovial fluid from baseline up to week 19, while remaining stationary in the contralateral right knee.

Conclusion:

The ACLT + pMMx model effectively induced a moderate to severe OA pathology in sheep after 27 weeks. The medial tibial condyle was the most impacted sub-region due to the removal of the cranial part of the medial meniscus during the surgical procedure. Most of the parameters investigated in this study (synovial fluid score, macroscopy of the cartilage, osteophyte and synovial membrane and the microscopy of the cartilage and synovial membrane) evidenced a clear damage of the left operated knee joint structures in comparison with their contralateral counterparts. Synovitis and cartilage degradation were further confirmed by increased total protein content and Coll2-1 levels in the synovial fluid of operated knees.

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