Evaluation of the synovial tissue revealed at 15 minutes post-injection countless numbers of intact RBC that were almost completely disappeared after 48 hours, with only limited recruitment of macrophages and iron deposition.

Conclusions: Blood is cleared very rapidly from the canine knee joint, but in that short time span already has adverse effects on both cartilage and synovial tissue. This rapid clearance can play a role in the discrepancy between long-term in vitro and in vivo effects of blood-induced joint damage since more than 10% of RBC blood for 48 hours is needed induced to long-term adverse effects in vitro. Irrespectively, blood has devastating effects on articular cartilage very rapidly, and in this respect it is important to prevent (traumatic) joint haemorrhages and if they occur, to treat them properly.

56 THE BMP ANTAGONISTS FOLLISTATIN AND GREMLIN IN NORMAL AND EARLY OSTEOARTHRITIS: AN IMMUNOHISTOCHEMICAL STUDY

G. Tardif, J-P. Pelletier, C. Boileau, J. Martel-Pelletier. Osteoarthritis Research Unit, University of Montreal Hospital Center, Notre-Dame Hospital, Montreal, QC, CANADA

Purpose: Bone morphogenic protein (BMP) activities are controlled in part by antagonists. In human osteoarthritic (OA) cartilage, the BMP antagonists follistatin and gremlin are increased, but differentially regulated. Using the OA dog model, we determined if these BMP antagonists were produced at different stages during the disease process by comparing their in situ temporal and spatial distribution.

Methods: OA was induced in dogs by sectioning the anterior cruciate ligament. Dogs were sacrificed 4 (n=4), 8 (n=4), 10 (n=5) and 12 (n=4) weeks after surgery, and non-operated (n=7) dogs served as normal controls. Cartilage was removed, differentiating fibrillated and non-fibrillated areas. In situ protein levels of follistatin, gremlin, BMP-2/4 and II-1 were determined by immunohistochemistry and morphometric analyses. Growth factor-induced gremlin expression was also assessed in dog chondrocytes by real-time PCR.

Results: Follistatin and gremlin production was very low in normal cartilage. Gremlin was already significantly up-regulated in both non-fibrillated (p < 0.004) and fibrillated (p < 0.02) areas at 4 weeks post-surgery, and only slightly increased with the progression of the disease. Follistatin showed a time-dependent increased level in the non-fibrillated areas with significance (p < 0.02) reached at 8–12 weeks; in the fibrillated areas high levels were already seen at 4 weeks with significance reached at each time point (p < 0.0001). In the whole cartilage, follistatin and II-1 temporal production showed similar patterns; this was also true for gremlin and BMP-2/4, although BMP-2/4 production was already high in the normal dogs. Data revealed that the basic fibroblast growth factor (bFGF) could be another factor that increases gremlin early in the disease. Indeed, bFGF markedly induced gremlin in dog chondrocytes. Moreover, and although a similar level of bFGF was found in both normal and OA cartilage, the presence of a marked positive staining in the extracellular matrix throughout the cartilage layers was found only in the OA cartilage. Comparison between superficial and deep zones revealed that follistatin is located mostly in the superficial zone of the cartilage, while gremlin is produced both in the superficial and deep layers. A similar pattern for follistatin and II-1 was found only in the superficial zone, whereas gremlin and BMP-2/4 had similar profiles in both zones.

Conclusions: Data show, for the first time, different spatial and temporal production of gremlin and follistatin in cartilage during the progression of OA. These data, in addition to the significant up-regulation of gremlin earlier than follistatin, strongly suggest that gremlin is involved during the early/remodeling phases, and follistatin linked to the inflammatory process of the disease. These findings may reflect different roles for each antagonist in this disease.

57 PHARMACOLOGICAL INTERVENTION ON SPONTANEOUS OSTEOARTHRITIS IN STR/OR T MICE: EFFECTS OF AGGRECANASE OR COX-2 INHIBITION

R. Chiusaro1, A. Giordani1, I. Verpilio1, S. Mandelli1, P. Ballanti2, M. Lanza1, G. Caselli1, L.C. Rovati1. I.Rottapharm S.p.A., Monza, ITALY, 2 Policlinico Umberto I, Roma, ITALY

Purpose: The STR/or mice develop spontaneous osteoarthritis (OA) of the knee, and are believed to be a relevant model for human knee OA. We have recently shown that treatment with glucosamine sulfate reduced the severity of the OA histological score as well as histomorphometric parameters in this strain. To further validate this model for drug discovery purposes, we investigated its responsibility to further interventions believed to be effective in either murine or human OA. While administration of the selective Cox-2 inhibitor celecoxib has been shown to be effective on the symptoms of human OA, it has been recently demonstrated that deletion of aggrecanase-2 protects mice from cartilage degeneration in surgically-induced OA. Therefore we investigated in two separate studies the effects of celecoxib or of a specific aggrecanase inhibitor (CR 4152), respectively, in STR/or mice.

Methods: STR/or male mice were recruited at 5 months of age (n=20–22). One group of animals was sacrificed at this age and served as a “baseline” control. Celecoxib (10 and 30 mg/kg b. w.) was administered orally once daily for 3 months, while CR 4152 (10 and 30 mg/kg of b.w.) was administered subcutaneously once daily for 3 months. At the end of treatment, the animals were euthanized and the knee joints collected, processed for histology and blindly scored according to both Mankin’s and the OARSI method. Furthermore, in the CR 4152 study, a histomorphometric analysis was performed on the medial tibial plateau, measuring the parameters of cartilage volume and lesion surface, with the operator still blinded to the experimental groups. Statistical analysis was performed by the Student’s t test or by ANOVA followed by Dunn’s or Dunnett’s tests comparing all treatment groups vs. vehicle.

Results: After three months of daily treatment, we observed that vehicle-treated animals displayed severe OA with clefting and erosion of the articular cartilage to the subchondral bone, with prominent chondro-osseous metaplasias and often inflammation and pannus. OA grade × stage score of the medial tibial plateau was significantly higher in vehicle vs. naïve 5-month (baseline) mice. In the celecoxib 30 mg/kg group there was only a trend for a decrease of the OA grade × stage score that was far from statistical significance (p = 0.648, Figure 1). Conversely, the OA grade × stage score was significantly decreased in the CR 4152 30 mg/kg group compared to vehicle (Figure 2).

Figure 1. OARSI scoring method (grade × stage, median); celecoxib group.