Introduction. Loss of the large aggregating proteoglycan aggrecan, from articular cartilage is a central pathological feature of joint diseases such as osteoarthritis (OA). Depletion of aggrecan from the cartilage as a result of proteolytic degradation occurs as an early event in disease progression and exposes the tissue to mechanical disruption. Matrix metalloproteinases (MMPs) and aggrecanase(s) are both believed to play a role in aggrecan turnover and increased degradation in pathology although the relative importance of these two distinct enzymatic activities in aggrecan catabolism in joint disease remains unclear. Development of therapeutic strategies for the management of OA relies on the use of models which mimic the naturally occurring disease. It is therefore paramount to determine the enzymes responsible for the cartilage degradation in the various animal models utilized. In the present study we have evaluated the temporal relationship between aggrecanase and MMP generated aggrecan catabolism in the rat iodoacetate model of OA.

Methods. Male Sprague Dawley rats (220-230 g) were injected intraarticularly in the left knee joint with 0.35mg of sodium iodoacetate in sterile saline. Groups of ten animals were sacrificed 0 (uninjected controls), 1, 3, 7, 14 and 21 days post injection. The left knees of sacrificed animals were disarticulated and the surface of the tibia was imaged using an Optimas image analyzer. The severity of damage in the magnified images was assessed by three independent observers in a blinded manner using a scale of increasing severity (0 = normal - 4 = maximum severity). The articular cartilage was placed in a mixture of guanidine and proteinase inhibitors for 48 hours at 4°C. Following extraction the cartilage residue was digested with papain and the proteoglycan content of the cartilage extract and papain digest was measured as sulphated glycosaminoglycan (GAG) using a dimethylmethylene blue assay. Total GAG in the cartilage samples (GAG in the extract + the digest) was express as µg/mg wet weight. The guanidine extracts were dialyzed and the proteoglycan fragments deglycosylated with chondroitinase ABC, keratanase and keratanase II prior to separation by 10% SDS-PAGE. Samples were loaded such that the extract from an equal wet weight of cartilage was run in each lane. Electrophoretically separated samples were transferred to nitrocelullose and immunolocated with antibodies recognizing the aggrecanase generated C-terminal aggrecan neoepitope _NITEGE_ (BC-13) and the MMP generated C-terminal aggrecan neoepitope _DIPEN_ (anti-DIPEN). Blots were digitized and the density of immunoblot bands quantified using NIH Image software and expressed as a percentage of the highest value. Quantitative data were compared using ANOVA with p < 0.05 being considered statistically significant.

Results and Discussion. There were virtually no gross pathological changes observed at days 1 and 3 post injection. However, joint lesions significantly increased on days 7, 14 and 21 compared to day 0 and there was a progressive increase in the pathology score on Days 7, 14 and 21. The articular cartilage GAG content decreased significantly with time. There was no change in GAG content on days 1 or 3, however there was a significant decrease in GAG content compared with Day 0 on Days 7, 14 and 21 (P < 0.001). The GAG content on Day 21 was significantly lower than all other days (P < 0.002). The change in GAG content was consistent with the gross pathology scores although it is of interest that the loss of GAG did not precede gross cartilage pathology. It may be that with time points between Day 3 and 7, decreased GAG may have been observed before gross changes in cartilage were observed.

A representative Western blot for NITEGE and DIPEN in cartilage extracts is shown in Figure 1. Two predominant NITEGE terminating G1 fragments were observed one at 70kDa and a second at 50kDa. While the 70kDa band was consistent with observations in other species the 50kDa NITEGE band has not been observed previously [1]. The 50kDa band must result from further proteolysis in the N-terminus of the G1 molecule without affecting the HA binding capacity as it is retained within the cartilage.

It is possible that there is a cleavage in one of the three disulphide bonded N-terminal loops, which does not affect HA binding. The small N-terminal peptide that is presumably removed to generate this 50kDa NITEGE catabolite is most likely disulphide bonded and part of an "intact" G1. However, as all of the gels are run under reducing conditions, this small peptide is released. The 50kDa NITEGE terminating fragment was present in the Day 0 samples and must have resulted from earlier catabolism and accumulation during the rats lifetime. While this fragment has been generated by aggrecanase cleavage of the IGD, the enzyme responsible for the further N-terminal clip is unknown. A single DIPEN terminating aggrecan metabolite (approx. 55 kDa) was observed in the rat articular cartilage (Figure 1). This metabolite is similar in size to that seen in other species.

![Western blot analysis for aggrecanase- (NITEGE) and MMP-generated (DIPEN) aggrecan metabolites in the articular cartilage 0-21 days after iodoacetate injection.](image-url)