Inhibition of cartilage erosion but not chondrocyte hypertrophy or osteophyte development in Mmp-13 knock out mice following surgical induction of osteoarthritis

INTRODUCTION

Cartilage destruction in osteoarthritis (OA) is driven by proteolysis of the principal matrix constituents, aggrecan and type II collagen. Aggrecanolysis precedes and may be prerequisite for collagenolysis, with the latter likely representing the point of irreversible structural damage. Members of the disintegrin and metalloprotease with thrombospondin motif (ADAMTS) family are primarily responsible for pathological aggrecan catabolism from cartilage. Mice with constitutively inactive ADAMTS-5, or mutation of the ADAMTS cleavage site in thrombospondin motif (ADAMTS) family are primarily responsible for joint disease in a surgical model of OA in mice. However, it is likely that at the time of clinical presentation, a patient will already have cartilage disease with aggrecan loss and in this situation it is unclear that inhibition of ADAMTS activity would prevent subsequent cartilage erosion. However, the relative contribution of MMP-driven aggrecanolysis versus physical disruption of aggrecan-depleted cartilage, to progressive cartilage erosion is unclear. In order to address this question, we examined the effect of Mmp-13 KO on the progression of joint disease in a surgical model of OA in mice.

METHODS

Medial meniscal destabilization was performed on the right knee of 10 wk old male Mmp-13 KO and wild type (WT) FVBN mice. Animals were housed in groups, received water ad libitum and were weighed weekly until sacrifice at 4 or 8 wks post-surgery (n = 10-15/genotype/timepoint). Knees were fixed in formalin, decalcified in formic acid and paraform embedded. Serial sagittal sections through the width of the medial femoro-tibial joint were cut. Sections every 40 μm were stained with toluidine blue and two observers blinded to genotype scored femoral and tibial cartilage proteoglycan (PG) loss (0-3) and erosion (0-7), with maximal and summed score recorded. Osteophytes of size (0-3) and maturity from predominantly cartilaginous to bone (0-3) were scored on digital images. The presence or absence of chondrocyte hypertrophy in non-calcified articular cartilage was noted and serial sections stained for collagen type X and the MMP-generated aggrecan neopeptid …DIPEN. All animal procedures were done in accordance with NHMRC guidelines under the approval of the institutional animal ethics committee (protocol no. 07/03-018A). Differences between genotypes or time post surgery were analysed by Mann Whitney U (non-parametric histological scores) or Chi-square (presence of hypertrophy) using Statview software, with p < 0.05 considered significant.

RESULTS

There was no difference in starting weight between genotypes and all animals gained weight equally over the course of the experiment. Meniscal destabilization induced PG loss and cartilage erosion in WT mice, summed scores being more severe in the tibia than femur at both time points (p<0.01). In WT mice, maximal and summed tibial cartilage erosion score but not PG loss score increased from 4 and 8 wks (p<0.05; Figure 1). There was no difference in PG loss or cartilage damage between KO and WT mice at 4 wks. In femoral cartilage, PG loss progressed from 4 to 8 weeks and was worse in KO at 8 wks (p<0.001), but structural damage did not differ from WT at either time. Unlike WT mice, tibial cartilage erosion did not progress with time in KO mice, and both maximal and summed score were significantly less in KO than WT at 8 wks (p<0.02; Figure 1).

Articular chondrocyte hypertrophy was evident in 70-90% of joints with no difference between genotypes or with post-operative time (p>0.3). Chondrocyte hypertrophy was associated with positive type X collagen and DIPEN staining in both WT and KO mice with no difference between genotypes.

In WT mice, cartilaginous osteophytes were present at 4 weeks and underwent endochondral ossification, with both size and maturity score increasing from 4 to 8 wks (p<0.01). At 4 weeks cartilaginous osteophytes were larger in KO than WT (p < 0.01), but by 8 weeks there was no difference in size or maturity score between genotypes.

DISCUSSION

These studies have confirmed that cartilage structural damage in OA in mice is dependent on MMP-13 activity and, at least in this mouse model with normal physiological loading levels, physical disruption of aggrecan-depleted cartilage did not lead to significant cartilage erosion. That cartilage erosion could be inhibited in the face of active ADAMTS-driven aggrecan depletion in Mmp-13 KO mice supports the potential for therapeutic intervention in established OA. We hypothesise that the more severe aggrecan loss in the femur of KO mice may have been a result of the absence of opposing tibial cartilage erosion with subsequent minimization the focal compression caused by the displaced meniscal tip. Again, this focal area of PG loss did not progress to structural cartilage damage/erosion in the absence of MMP-13.

We observed focal chondrocyte hypertrophy in non-calcified articular cartilage following surgery. However, this occurred equally in KO and WT mice, with equivalent collagen X expression and MMP-cleavage of aggrecan, suggesting that in itself chondrocyte hypertrophy is not a cause of cartilage degradation, but the associated MMP-13 expression is critical. The equivalent cell-associated DIPEN generation in WT and Mmp-13 KO mice in articular cartilage and growth plate, suggests that this is associated with an alternative enzyme such as MMP-14. The larger cartilaginous osteophytes at 4 weeks in KO mice is consistent with delayed endochondral ossification. Ultimately the equivalent osteophyte development in both genotypes demonstrates that this process it is not linked with articular cartilage damage, but other factors such as joint instability in this model.


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