INTRODUCTION

Osteoarthritis (OA) is a common pathology that occurs in a wide range of species in a number of articular joints, although the prevalence between joints varies. Epidemiological studies have shown that OA is more prevalent in the human knee than ankle and that the equine metacarpophalangeal (MCP) joint has more osteoarthritic lesions than the proximal interphalangeal (PIP) and distal interphalangeal (DIP) joints. It is important to understand why only certain joints succumb to degeneration. One simple explanation often offered is due to the differences in their joint anatomy and biomechanical loading pattern. However, the mechanism appears to be more complex as several studies in humans demonstrate that these joints also differ in the molecular composition of their cartilage, metabolic activity, and sensitivity to catabolic stimuli, such as interleukin-1. It is therefore more likely that the reason for the joint-specific nature of OA is a combination of different loading patterns, composition and turn-over between the joints and with age. Accordingly, we have explored the hypothesis that the molecular composition, metabolic activity and sensitivity of equine cartilage from joints (MCP, PIP and DIP joints) that experience greatly varied loadings, is joint-specific.

MATERIALS AND METHODS

Macroscopically normal MCP, PIP and DIP joint cartilage from the left forelimb of thoroughbred-types was prepared from abattoir horses with no history of joint pathology. These were divided into two groups: Group A, Young adults; Group B, Aged adults. 7 horses for metabolic (Group A, n=4, 5.75 ±0.3 yrs; Group B n=3, >18 yrs) and 18 for composition (Group A n=12, 3.1 ±1.0 yrs; Group B n=6, >18 yrs) were analysed. Diced cartilage was randomized and pulse-labelled for up to 6 hours in DMEM supplemented with 1% foetal bovine serum, penicillin/streptomycin and 50µCi/ml [35S]-sulphate. For pulse-chase, cartilage explants were pulse-labelled for 5 hours with 50µCi/ml [35S]-sulphate and 90µCi/ml L-[4,5-3H]Leucine, then in fresh DMEM without isotope for up to 7 days. Media and cartilage extracts were analysed for total and labelled glycosaminoglycans (GAG) and protein using GAG and BCA assays. Total and labelled cartilage oligomeric matrix protein (COMP) was quantified by ELISA and an antibody-stephalococcus A pull-down assay, respectively. For composition analysis, total content of water, protein, GAG, DNA, COMP and fibronectin were quantified.

RESULTS

There was a small but significant difference in the hydration level of the cartilage of the three joints, with the MCP joint having the lowest water content (approximately 5% less, p≤0.005) compared to the other two joints. This did not alter with age, indicating that the hydration of cartilage remains remarkably constant with age. This contrasted with the GAG and total protein content which was significantly higher in the DIP joint (1.5 times more) than the MCP or PIP joints in both age groups (p≤0.005), but the Aged horses had significantly more GAG (1.3 times more) in all the joints than the Young horses (p≤0.005), suggesting that the GAG levels per cell increases with age. The COMP levels were only significantly different between the joints of the Aged horses where the MCP had significantly less (3.5 times) than the PIP or DIP joints (p≤0.005). There was a 2-fold increase between the Young and Aged horses for the PIP and DIP joints (p≤0.005). Western blot confirmed significant levels of both COMP and fibronectin degradation fragments accumulated in both age groups but there were no differences with age. Pulse-labelling experiments showed that the MCP cartilage rapidly incorporated label and reached a maximum at 3 hours for both age groups, although the incorporation was significantly lower in the Aged horses (p≤0.05). The other two joints incorporated label linearly up to 6 hours in the Young group but remained at significantly lower rates than the MCP joint for either groups (p≤0.0001). The Aged group appeared to plateau after 3 hours although there was no significant difference between the two groups. Pulse-chase analysis to ascertain the turnover of matrix components, showed that the amount of labelled GAG release into the media was significantly higher in the MCP than the distal joints (p≤0.05) and the DIP joint had significantly less label in the explant than the MCP or PIP joints (p≤0.05). The MCP was the only joint to be significantly affected by age with less released with age into the media over the 7 days chase (p≤0.0001) (Figure 2). The labelled COMP released in both age groups was similar and increased significantly in the media (p≤0.0001) from day 0 to 7, which was the same as the GAG distribution. COMP released into the media was significantly higher in the MCP than the distal joints (p≤0.05).

DISCUSSION

Our study has utilized a model of articular cartilage derived from three joints that are anatomically in close proximity but experience remarkably different mechanical loads during locomotion. The MCP joint is a high load, high motion joint that has the largest number of site specific traumatic and degenerative lesions. The incidence rate of MCP OA is 31%, compared to 12% for PIP and DIP combined. Our data confirms the hypothesis that the articular cartilage from various joints has very different biochemical composition and metabolic behaviour. Thus, the MCP joint has a higher synthesis rate combined with a shorter half-life for GAG and COMP. The MCP cartilage has a GAG half-life of 3 hours whereas it is at least twice as long in PIP and DIP cartilage. This is an interesting finding as in the human knee and other equine cartilages this plateau is attained after 6 hours suggesting that MCP cartilage incorporates GAG much faster, and also degrades it faster. It would be interesting to determine if newly synthesised COMP is assembled into its mature pentameric form prior to release from the extracellular matrix, as it would give an indication of the ability of cartilage to turnover/assemble the extracellular matrix in an effective manner. Studies have shown human chondrocytes can synthesise and release pentameric COMP in culture. MCP cartilage is unique as the only joint significantly affected by age, with a reduction in new synthesis. In conclusion, MCP cartilage has a quick turnover, but there appears to be an optimised balance between synthesis and degradation. This is reflected in the total GAG which remained unchanged. What this does suggest, however, is that even a minor perturbation in this rapid control of homeostatic balance would quickly tip the scales in the favour of synthesis or degradation. Higher prevalence of OA in MCP cartilage would suggest that this imbalance is often in favour of degradation. Turnover of COMP further supports the notion that specific compartments are subject to preferential degradation, causing localised or focal loss of cartilage integrity, which is a hallmark of OA lesions.

REFERENCES


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