Differential Effects of Canine Cartilage to Cytokine Stimulation and N-3 Polyunsaturated Fatty Acid Supplementation Versus Human and Bovine Cartilage

Introduction
Arthritis is a significant problem in both humans and animals that may occur at any age but is particularly common in older individuals. In dogs, both inflammatory and degenerative joint disease can occur, but the most common form observed is osteoarthritis (OA). Previous work in our laboratory has shown that supplementing human OA cartilage and bovine cartilage with n-3 polyunsaturated fatty acids (PUFAs) can inhibit the degenerative and inflammatory processes observed in OA [1,2]. This present study was designed to evaluate whether supplementation of canine articular cartilage with n-3 PUFAs would result in an observed reduction in degeneration and inflammation that is a hallmark of canine OA.

Materials and Methods
Full-depth articular cartilage was harvested from the knee/stifle joints of dogs that were euthanized for other medical reasons. Cartilage was harvested from 11 dogs (5 female and 6 male), with an age range of 11.01-14.98 (average age of 13.49). Cartilage explant pieces were pre-cultured in DMEM containing 10% FCS for 48 hours. Following this time, explants were washed and incubated in the presence of n-3 PUFAs (EPA, DHA and α-linolenic acid) for 5 further days to allow incorporation of the n-3 PUFAs into the cartilage. After 5 days, explants were washed in order to remove the fatty acid, and they were cultured for a further 4 days in the presence of oncostatin M (OSM; in order to induce a degradative phenotype). At the end of the culture period, GAG release was measured using the DMMB assay, and lactate was measured in order to study effects on cartilage metabolism. Western blot analysis was carried out as previously described [1,2] using antibodies BC-3 (to the aggregcanase generated neoepitope) and BC-14 (to the MMP generated neoepitope). RT-PCR analysis was also performed as previously described [1,2].

Results
Addition of OSM to canine explant cultures resulted in an increase in GAG release (Figure 1), with a concomitant increase in BC-3 immunoreactivity (aggrecanase) in canine cartilage explants (representative blot from one dog).

Discussion and Conclusion
Previous studies in our laboratory showed a decrease in degradative and inflammatory factors in human OA and bovine cartilage supplemented with n-3 PUFAs. Interestingly, we observed similar effects in canine articular cartilage, although there were some interesting and significant differences between the systems. Firstly, in our human OA and bovine studies, in order to reduce degeneration, it was necessary to supplement with the n-3 PUFAs for 24 hours. In contrast, in canine cartilage, before any beneficial effect was observed, it was necessary to supplement with the n-3 PUFAs for 5 days (120 hours). Secondly, in our human OA and bovine studies, all of the n-3 PUFAs (EPA, DHA and α-linolenic acid) had an equally beneficial effect. In contrast, in canine cartilage, only EPA had a beneficial effect on the degradation seen. Thirdly, in order to induce degradation in the bovine system IL-1α was used, whereas in canine cartilage IL-1α has no effect on GAG release and so OSM was used.

In conclusion, this study shows that there is a beneficial effect of n-3 PUFAs (specifically EPA) on the OSM induced degradation seen in canine articular cartilage. Similarly, improvements in arthritic signs in dogs fed green-lipped mussel (contains high n-3 PUFA content) has been reported recently [3]. These data advocates further study into the possible beneficial effects of n-3 PUFAs in canine OA.

References

FIGURE 1: Effect of n-3 PUFAs supplementation on GAG release in canine cartilage explants (mean ± SD, n=11)

FIGURE 2: Effect of n-3 PUFAs supplementation on BC-3 immunoreactivity (aggrecanase) in canine cartilage explants (representative blot from one dog)

There was no BC-3 immunoreactivity in control cultures. Analysis of the BC-3 immunoreactivity in OSM treated explants indicated a split of the dogs into two groups. 8 out of the 11 dogs tested showed a decrease in GAG release on the addition of EPA and a concomitant decrease in BC-3 immunoreactivity (Figure 2) at the higher concentration tested (300μg/ml). In contrast, the remaining 3 dogs showed a decrease in GAG release with the addition of EPA, whilst having little or no effect on the BC-3 immunoreactivity. Addition of DHA resulted in a decrease in BC-3 immunoreactivity in all of the dogs (Figure 2), but as indicated earlier, this was most likely due to a decrease in metabolism of the canine cartilage as indicated by a decrease in lactate. In contrast, in all of the dogs, α-linolenic acid had no effect on the GAG release and similarly had no effect (or resulted in an increase) in BC-3 immunoreactivity. RT-PCR analysis of RNA extracted from the fatty acid supplemented explants showed that supplementation with any of the n-3 PUFAs had no effect on the expression of the aggrecanases (ADAMTS-4 and –5) or MMP-3 and –13.

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