Kinetics of release of aggrecan from explant cultures of bovine cartilage from different sources and from animals of different ages

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It is the presence of aggrecan trapped as a complex with hyaluronan and link protein within the collagen fibres that gives articular cartilage its unique biomechanical properties of being able to withstand compressive loads (Maroudas 1980). The chondrocytes are responsible for the synthesis and catabolism of aggrecan complexes in the extracellular matrix of cartilage. In normal tissue the concentration of aggrecan complexes in the extracellular matrix is maintained at a constant level (Handley et al. 1990). It has been shown that when articular cartilage is exposed to retinoic acid or interleukin-1 there is an elevated rate of loss of aggrecan from the tissue (Ilic et al. 1992). Work utilising explant cultures of cartilage has shown that the catabolism of aggrecan in cultures exposed to either retinoic acid or interleukin-1 involves the cleavage of the core protein of this proteoglycan at a distinct site within the interglobular domain (Sandy et al. 1991, Ilic et al. 1992). Little is known about the kinetics of loss of aggrecan from cartilage and how this varies with the source of tissue and the age of the animal that the tissue is derived from. This paper describes the kinetics of loss of both the chemical and radiolabelled pools of aggrecan present in explant cultures of bovine articular and nasal septum from foetal and mature animals maintained in the presence and absence of retinoic acid.

Materials and methods

All materials were as described previously (Ilic et al. 1992).

Cartilage was isolated under sterile conditions from metacarpalphalangeal joints or nasal septum of mature (2–3 year old) or foetal cattle. The tissue was cut into pieces, approximately 2 mm^3, and placed in culture in a modification of Dulbecco's modified Eagle's medium containing 20% (v/v) new-born-calf serum (Hascall et al. 1983). The medium was changed every 24 h.

After 5 days in culture, the tissue was incubated with medium containing 20% (v/v) new-born-calf serum and [^{35}S]-sulphate (20 mCi/ml) for 6 h. At the end of the incubation period, the tissue was washed three times in medium alone, divided into duplicate 100 mg lots and replaced in culture for a further 7 day period in vials containing 4 ml of medium alone or medium containing 10^{-6} M retinoic acid. Medium was changed daily and the spent medium stored at –20 °C in the presence of protease inhibitors (Ilic et al. 1992). Cultures were extracted with 4.4 ml of 0.5 M NaOH immediately after incubation with radiolabelled sulphate or at the end of the culture period. The extraction was carried out at room temperature over a period of 24 h.

The amount of glycosaminoglycans present in the matrix of cartilage explants on day 5 and 12 of culture and appearing daily in the medium of the explant cultures was determined by analysing aliquots of the NaOH extracts of the tissue and spent medium for sulphated glycosaminoglycans using the dimethylene blue dye binding assay as previously described (Farndale et al. 1986). Chondroitin sulphate prepared from bovine nasal septum cartilage was used as standard. To determine the amount of ^{35}S-radioactivity associated with proteoglycans present in the matrix of the explant cultures or lost to the medium on each day, aliquots (0.5 ml) of the spent medium or NaOH tissue extracts were subjected to size-exclusion chromatography on columns (1.5 x 5.5 cm) of Sephadex G-25. These data were used to determine the amount of radiolabelled macromolecules appearing in the medium on each day and the half-life of ^{35}S-labelled proteoglycans present in the extracellular matrix of the explant cultures (Ilic et al. 1992).

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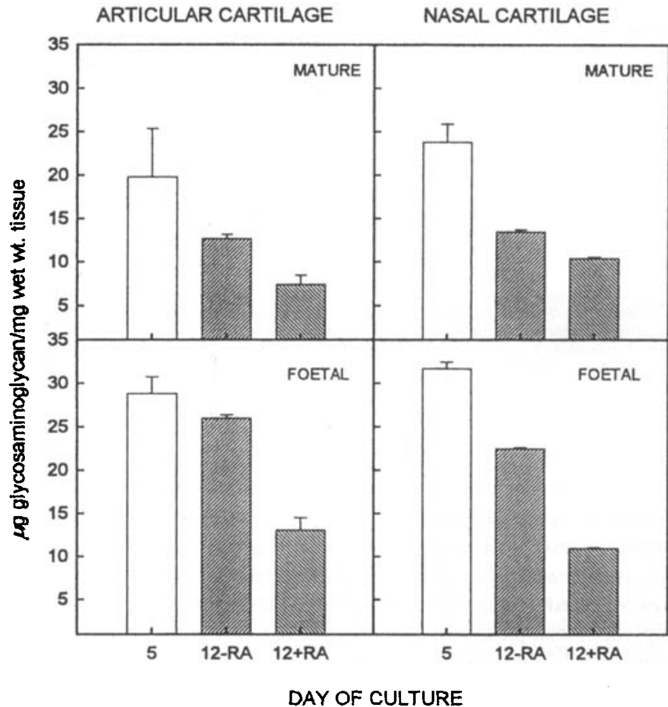

Figure 1. The glycosaminoglycan content of explant cultures of articular and nasal cartilage from mature and foetal cattle maintained in explant culture in medium alone or medium containing retinoic acid. The levels of glycosaminoglycans present were determined in explants of cartilage maintained in medium containing 20% (v/v) new-born-calf serum for 5 days (5) and in tissue maintained in medium alone (12–RA) or medium containing 10^{-6} M retionic acid (12+RA) for a further 7 days.

Results and Discussion

The tissue levels of sulphated glycosaminoglycans present in the extracellular matrix of explant cultures of articular and nasal cartilage from mature and foetal cattle are shown in Figure 1. It is evident that when cartilage was placed in culture in medium containing retinoic acid there was a marked decrease in the tissue levels of glycosaminoglycans irrespective of the source of cartilage or the age of animals. When cultured in medium alone there was also a decrease in the tissue levels of glycosaminoglycans but not to the same extent as that observed in tissue exposed to retinoic acid. It was also apparent that there was a greater loss of glycosaminoglycans in cartilage explants derived from mature animals.

When articular cartilage from mature and foetal cattle and nasal septum cartilage from foetal animals was placed in culture in medium alone there was a constant low rate of appearance of glycosaminoglycans in the medium over the culture period (Figure 2). This indicates that in cartilage maintained in medium alone, the decrease in tissue glycosaminoglycan levels observed in Figure 1 was most likely to be the result of a decrease in the rate of synthesis of aggrecan due to an absence of growth factors such as insulin-like growth factor-I in the culture medium (McQuillan et al. 1986). When exposed to retinoic acid there was an increase in the rate of appearance of glycosaminoglycans in the medium of these explant cultures. The rate of appearance of glycosaminoglycans reached a maximum 2–3 days after exposure to retinoic acid and then fell to levels comparable to cultures maintained in medium alone. The rate of appearance of glycosaminoglycans in the medium was greater in nasal and articular cartilage from foetal animals. These data along with that described in Figure 1 are consistent with previous observations which showed that when cartilage is exposed to retinoic acid there is a stimulation in the catabolism of aggrecan (Ilic et al. 1992). Analysis of the aggrecan fragments appearing in the medium of explant cultures of articular and nasal cartilage from mature cattle exposed to retinoic acid have shown that this increase in the catabolism of aggrecan involves the cleavage of the core protein at a specific site within the interglobular domain corresponding to glu(392) and ala(393) in the cDNA sequence of the human aggrecan core protein (Ilic et al. 1992, unpublished data). This cleavage site has been described as the aggrecanase site, after the putative proteinase that is responsible for the cleavage of the aggrecan core protein. Previous work using an inhibitor to metalloproteinases has indicated that the protease responsible for the cleavage of aggrecan at this site is a metalloproteinase that can be activated by other proteases

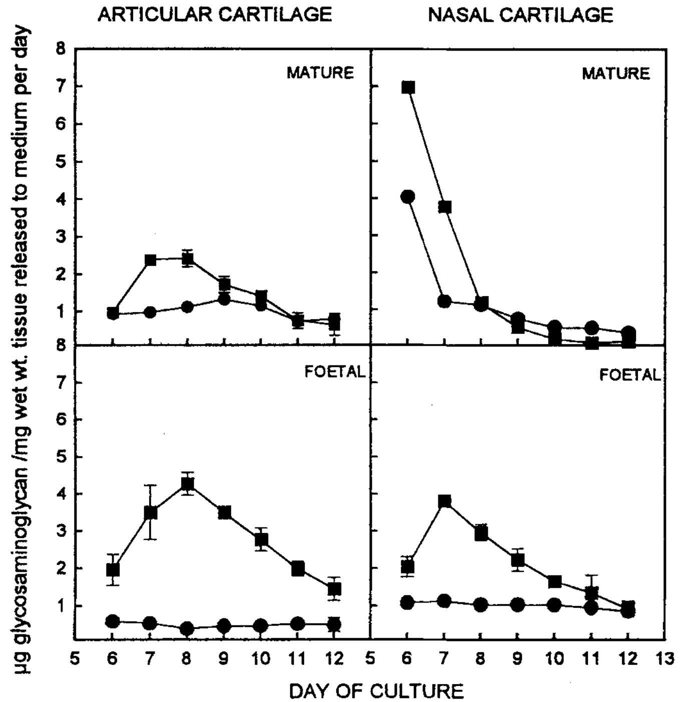

Figure 2. The appearance of glycosaminoglycans in the medium of explant cultures of articular and nasal cartilage from mature and foetal cattle maintained in explant culture in medium alone or medium containing retinoic acid. Cartilage was maintained in medium containing 20% (v/v) new-born-calf serum for 5 days and then cultured in the presence of medium alone (●) or medium containing 10^{-6} M retinoic acid (■) for a further 7 days. The levels of glycosaminoglycans appearing in the medium on each day was determined.

such as cathepsin B (Buttle et al. 1994). Recent work has shown that retinoic acid stimulates the expression of mRNA coding for metalloproteinases in chondrocytes (Ballock et al. 1994). The 2–3 day lag before the maximum rate of catabolism of the chemical pool of aggrecan could be due to a time dependent induction of the protease or an indication of the time taken for this protease to move into the inter-territorial matrix of the tissue. It is also possible that this protease is present as a latent enzyme within the interterritorial matrix and the lag is due to its activation. When cartilage from the nasal septum from mature animals was placed in culture in medium alone or medium containing retinoic acid there was an immediate elevated appearance of glycosaminoglycans in the medium which declined with the time of culture.

The amount of ^{35}S-labelled proteoglycans appearing in the medium on each day is shown in Figure 3. The kinetics of appearance of ^{35}S-labelled aggrecan observed in cultures maintained in medium alone was similar to the appearance of chemical levels of this proteoglycan. In cultures of articular cartilage from mature cattle maintained in medium alone, the proportion of the radiolabelled pool of aggrecan lost from the tissue was significantly greater than that observed in articular cartilage from foetal animals. When cultured in the presence of retinoic acid there was stimulation in the appearance of radiolabelled proteoglycan in the medium of these cultures. In the case of articular cartilage there was an immediate stimulation in the appearance of ^{35}S-labelled aggrecan in the culture medium which was different to that observed for the chemical pool where a maximum rate of appearance of aggrecan was observed 2–3 days after exposure to retinoic acid. In foetal nasal septum cartilage the kinetics of appearance of the radiolabelled aggrecan was similar to that observed for the chemical pool of aggrecan where a maximum was observed after 2–3 days exposure to retinoic acid (Figure 2). In nasal cartilage from mature animals there was an immediate rapid appearance of radiolabelled proteoglycan in the culture medium that was marginally stimulated in cartilage cultured in the presence of retinoic acid. Again the kinetics of loss was similar to that observed for the appearance of the chemical pool of proteoglycan in the culture medium. Using the data shown in Figure 3, the half-lives of the radiolabelled pool of aggrecan were calculated (Ilic et al. 1992); for explant cultures of articular and nasal septa cartilage from foetal animals values of 29 and 31 days respectively were obtained for tissue maintained in medium alone and 3 and 2.5 days for tissue exposed to retinoic acid. Half-lives of 10 and 3 days were measured for proteoglycan in explant cultures of articular cartilage from mature cattle maintained in medium alone and medium containing retinoic acid.

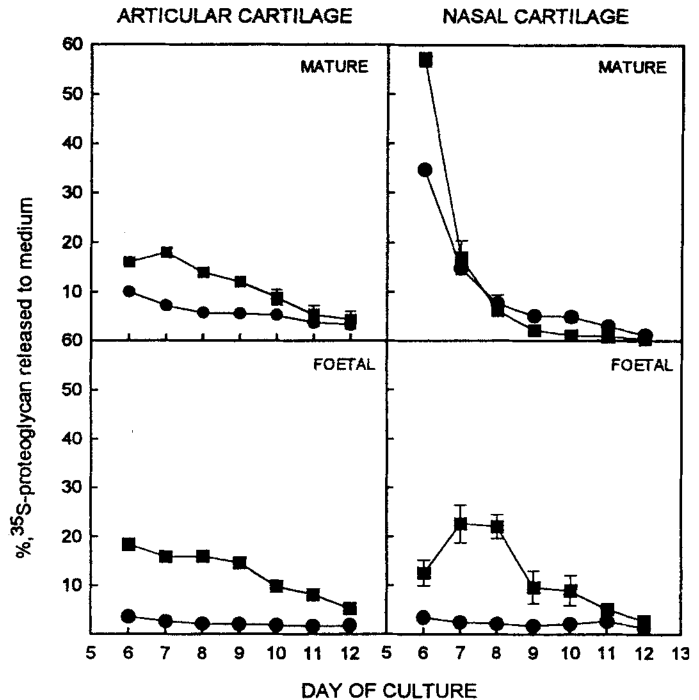

Figure 3. The appearance of ^{35}S-labelled proteoglycans in the medium of explant cultures of articular and nasal cartilage from mature and foetal cattle maintained in explant cultures in medium alone or medium containing retinoic acid. Cartilage was maintained in medium containing 20% (v/v) new-born-calf serum for 5 days, incubated with [^{35}S]-sulphate for 6 h and then cultured in the presence of medium alone (●) or medium containing 10^{-6} M retinoic acid (■). The amount of ^{35}S-labelled proteoglycans appearing in the medium on each day was determined.

For nasal septum cartilage from mature animals, half-lives of 2 and 1 day were obtained for tissue maintained in medium alone and in medium containing retinoic acid.

Conclusions

This work shows that in explant cultures of articular cartilage derived from mature and foetal cattle exposed to retinoic acid, the kinetics of release of the chemical and radiolabelled pools of aggrecan are different. If it is assumed that the radiolabelled pool of aggrecan is located close to the chondrocytes within the pericellular matrix, the effect of retinoic acid is to stimulate the chondrocyte to express or activate the protease responsible for aggrecan catabolism resulting in this pool of aggrecan initially being degraded. The degradation of the chemical pool of aggrecan present in the territorial matrix is likely to require the protease to move into the territorial matrix or if the protease is present as an inactive enzyme within the territorial matrix there is a time dependent activation. The behaviour in explant culture of cartilage from nasal septum from mature cattle was striking in that both the radiolabelled and chemical pools of aggrecan were rapidly lost from the tissue irrespective of the presence of retinoic acid in the culture medium. This suggests that mature nasal cartilage may contain a greater potential to degrade aggrecan and this may be due in part to collagen network of this cartilage being more open and looser than that observed in articular cartilage (Blackwell, 1995). This observation also raises the question how is the catabolism of aggrecan in nasal cartilage regulated in vivo.

Acknowledgments

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