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The effect of age on proteoglycan aggregation in human cartilage

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Keywords

Aggrecan, aging, cartilage

Context

Aggrecan is the major proteoglycan in cartilage responsible for imbibing water and giving the tissue its ability to withstand compressive forces. As cartilage ages, these properties are lost and the risk of developing degenerative arthritis increases. It is known that catabolic post-translational modifications of aggrecan occur in the matrix during aging. For example, the number and size of the chondroitin and keratan sulphate chains decrease. It has also been shown that the rate of aggrecan synthesis decreases with aging. It has been observed that there is a delay between release and aggregation of aggrecan in the extracellular matrix. This appears to be dependent on maturation of the G1 (hyaluronan-binding) domain of aggrecan and the presence or absence of link protein (responsible for facilitating this binding). This paper examines the effect of age on the process of aggregate formation in human articular cartilage.

To determine whether age is an important factor in the rate and extent of aggregation of newly synthesised aggrecan in human cartilage.

Significant findings

In older cartilage, approximately 80% of the newly synthesised aggrecan was readily extracted by PBS and therefore presumed to be non-complexed with hyaluronan even after a 6 h chase. However, in immature cartilage, newly synthesised aggrecan after a 6 h chase required extraction by GnHCl, implying that it had already formed aggregates within a the tissue. Pulse-chase studies up to 15 days showed that slow incorporation of proteoglycan into the 4 M GnHCl extracted pool did occur in older cartilage but it did not reach the levels obtained by very young cartilage.

Analysis of combined PBS and GnHCl extracts by associative gel filtration chromatography showed that in young cartilage 60% of total sulphated proteoglycan was able to aggregate after a 1 h pulse compared to 31% in older cartilage. This did not appear to be dependent on the GnHCl fraction, as PBS extracts from young cartilage were able to aggregate under associative conditions.

The ability of exogenous hyaluronan and link protein to improve aggregation from the PBS extract of older cartilage was assessed. Although both hyaluronan and link protein increased aggregation, this could be partly inhibited by hyaluronan oligosaccharides (which the authors conclude signifies a low-affinity interaction). This experiment was not performed on immature cartilage.

Combined PBS and 4 M GnHCl extracts dialysed in associative conditions were analysed by agarose/PAGE. In young cartilage aggrecan was retarded and failed to enter the gel (ie it had aggregated) after a one day chase. This effect was partly inhibited by the addition of hyaluronan oligosaccharides, but was stabilised by the addition of link protein. In older cartilage little proteoglycan was retarded and hyaluronan oligosaccharides completely inhibited any aggregation. The addition of link protein resulted in formation of aggregates.

Comments

This paper addresses important questions regarding the effect of aging on normal human cartilage. The authors suggest that secreted aggrecan is processed very differently in young compared to old cartilage. They speculate that maturation of aggrecan is required before stable aggregation can occur, and that this process is delayed in older cartilage. However, this delay in aggregation could be explained by reduced synthesis of link protein, which has been demonstrated previously by the same group (Bolton et al, Biochem J 1999, **337:**77-82 Abstract). A key experiment to compare the affinity of aggregation (in the presence of exogenous link protein) in old versus young cartilage is missing.

Methods

Human cartilage was obtained from knee joints obtained from amputations. Explants were dissected, diced and randomised. Proteoglycan synthesis was measured by the incorporation of [^{35}S] sulphate. Rates of aggregation of aggrecan were determined by 'pulse-chase' experiments. Proteoglycan was extracted from explants by sectioning the samples at 20 ?m (in order to overcome diffusion difficulties), then extracting overnight in PBS alone, (to extract aggrecan not complexed with hyaluronan) or PBS followed by 4 M guanidine HCl (GnHCl, to extract aggrecan presumed to be complexed with hyaluronan). Gel filtration chromatography (Sepharose CL2B) was used to determine amounts of aggregated and non-aggregated aggrecan. Aggregation was also assessed using agarose/polyacrylamide gel electrophoresis. Autoradiography was used to assess distribution of newly synthesised aggrecan in tissue sections. Hyaluronan oligosaccharides were prepared by digesting hyaluronan with hyaluronidase and link protein was isolated from a porcine aggregate preparation.

References

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