

Keratan sulfate in body fluids in joint disease

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Keratan sulfate (KS) is a highly-negatively charged glycosaminoglycan which is found principally in aggrecan, the most abundant proteoglycan in the extracellular matrix of hyaline, fibrous and elastic cartilage. In 1985, we discovered that small peptidoglycans bearing antigenic KS (agKS) were present in small amounts in human serum and postulated that the great majority of these were derived from the degradation of cartilage aggrecan (Thonar et al. 1985). During the past decade, studies of antigenic KS in joint fluid and serum have provided strong evidence in support of this hypothesis. By asking questions which could not have been addressed previously, these studies have shed new light upon the metabolism of aggrecan in vivo. In this manuscript, we describe some of these findings and put in perspective their contribution to our understanding of the metabolism of aggrecan in joint diseases.

Composition and turnover of molecules bearing antigenic KS in tissues and body fluids

Most attempts to quantify KS in body fluids have used the well-characterized 1/20/5-D-4 anti-KS monoclonal antibody in a competitive indirect inhibition enzyme-linked immunosorbent assay (ELISA) (Thonar and Glant 1992). This antibody is directed against a highly sulfated sequence consisting of several repeats of the disaccharide {sulfated N-acetylglucosamine – sulfated galactose} present in long KS chains (Thonar et al. 1994). The ELISA provides a reliable measure of KS present in aggrecan from adult cartilage: the ratio agKS / total KS in human aggrecan remains fairly constant after 16 years of age (Fukuda et al. 1993). Further, the ELISA is as reliable in quantifying agKS in intact aggrecan as in small

peptidoglycans (Thonar and Glant 1992). Indeed, pretreatment of aggrecan with keratanase (*Pseudomonas* sp.), an enzyme which produces protein-free agKS oligosaccharides, does not cause a measurable decrease in antigenicity when the assay is performed at pH 5.3 as recommended (Thonar and Glant 1992). Interestingly, some of these agKS oligosaccharides contain two or more epitopes, each capable of binding to a separate antibody molecule in a sandwich ELISA (Shimozuru et al. 1995). As agKS is present in the body fluids of most vertebrate species (mouse and rat being among the exceptions), measurement of agKS in body fluids can be used to monitor changes in the rate of catabolism of aggrecan in a wide variety of animal models of joint disease.

Cleavage of the core protein of aggrecan by aggrecanase and/or stromelysin, the enzymes most likely responsible for the degradation of aggrecan in cartilage, produces one large fragment which contains most of the KS chains and essentially all of the chondroitin sulfate chains originally present on the intact aggrecan molecule (Lohmander et al. 1993). This large fragment is not found in significant amounts in the articular cartilage matrix and, consequently, most likely probably rapidly diffuses out of the tissue (Thonar et al. 1994). Measurement of agKS in joint fluid (i.e. synovial fluid or synovial lavage) thus offers a good measure of the rate of aggrecan degradation in articular cartilage within that joint. As all cartilaginous tissues in the body contribute to the pool of agKS-bearing peptidoglycans present in blood (Thonar et al. 1994), the concentration of agKS in serum or plasma provides a measure of the average rate of aggrecan degradation in the cartilaginous tissues in the body.

There is some evidence that only a minor proportion of the agKS-bearing fragments present in joint fluid reach the blood via the thoracic duct (Thonar et

al. 1993). It is possible therefore that most of the agKS-bearing fragments detected in blood enter this body fluid by diffusion through the membrane of capillaries lying in tissues that are in close proximity to cartilaginous structures. From the blood, the fragments are cleared by the liver and/or kidneys (Thonar et al. 1994). The rate of clearance from the blood is dependent upon the size and composition of the fragments: the subpopulation of agKS-bearing molecules which predominates in blood has a half-life of approximately 45 minutes (Thonar and Glant 1992).

Control of aggrecan synthesis and turnover

The turnover of aggrecan is believed to be conservative, i.e. entire aggrecan molecules are catabolized while those remaining in the matrix are left intact (Mok et al. 1994). The results of a recent *in vitro* study of the turnover of aggrecan in different matrix pools are consistent with the view that most agKS-bearing molecules present in body fluids are derived from aggrecan molecules degraded in close proximity to the cell membrane (Mok et al. 1994). The mechanisms which regulate turnover are under tight control since serum levels of agKS fluctuate very little from day to day and even from year to year, in normal adults as well as in patients with osteoarthritis (OA) (Thonar et al. 1993).

Measurement of agKS in joint fluid and serum prior to and after systemic administration of a drug can help determine if the medication has an effect upon the metabolism of cartilage aggrecan *in vivo*. Nonsteroidal anti-inflammatory drugs which have pronounced effects on the metabolism of articular cartilage *in vitro* have little, if any, effects on the serum level of agKS (Thonar et al. 1991). In contrast, oral administration of prednisone in human adults with asthma causes a rapid drop in the serum level of agKS (Thonar and Glant 1992). Importantly, the level of agKS in these individuals does not return to its pre-operative value until more than a month after cessation of treatment. In OA patients, a single intraarticular injection of prednisone causes the level of agKS to drop markedly not only in fluid obtained from that joint but in serum as well, strongly suggesting that the drug, even when injected locally, is able to exert its effects upon other cartilaginous structures in the body (Thonar and Glant 1992).

Metabolism of aggrecan in OA

agKS in synovial fluid

Posttraumatic OA of the knee offers an attractive model for the study of preradiological OA as the onset of the disease processes can be identified as the time of trauma. The destabilization of a knee following injury to a ligament or meniscus leads within hours to a marked increase in the concentration of aggrecan core protein epitopes in joint fluid (Lohmander et al. 1993). The level of these epitopes reaches a peak after approximately 1 week before declining slowly. The concentration of these aggrecan fragments, obtained by measuring levels of aggrecan core protein-related epitopes (Lohmander et al. 1993) or agKS (Thonar et al. 1993), remains elevated for several years. Importantly, these changes are not usually seen in individuals who present with knee pain without evidence of damage to at least one internal supporting element (Lohmander et al. 1993). The increase in aggrecan catabolism in a traumatized knee joint is believed to be balanced, at least at first, by an increase in aggrecan synthesis. This local state of chondrocyte hypermetabolism also leads to changes in both the anabolic and catabolic pathways of the metabolism of other matrix molecules, including collagen type II (Thonar et al. 1993). Future studies will hopefully attempt to determine whether and, if so, which of these metabolic changes predispose articular cartilage to OA in these joints.

Antigenic KS is present in elevated amounts in synovial fluid from human OA joints (Shimozuru et al. 1995). The levels of this and other markers of aggrecan catabolism are highest during the pre-radiological stages of the disease and tend to drop with time, especially in joints exhibiting secondary inflammatory changes or loss of articular cartilage mass (see Thonar et al. 1994 for review). This makes interpretation of the data difficult. However, this difficulty can be circumvented by measuring additional markers and reporting the results as ratios of one marker to another: this approach holds great promise and should provide significant new knowledge.

agKS in serum

Transection of the anterior cruciate ligament in a dog knee gives rise to a measurable increase in the serum level of agKS (Manicourt et al. 1991). This increase, which does not develop following a sham-operation, probably reflects changes occurring systemically rather than only in the injured joint. Indeed, comparison of the changes which take place in serum and joint fluid have demonstrated that the rise in serum reaches a peak later (i.e. 3 weeks vs 7 days) and

remains at this peak level longer (> 13 weeks vs < 5 weeks) than in joint fluid (Thonar et al. 1994). As most of the agKS in serum originates from non-articular cartilage, the elevation in serum most likely reflects the presence of a systemic state of hypermetabolism involving cartilaginous structures throughout the body (Thonar et al. 1994). These findings suggest that it is inappropriate to use the contralateral joint as a "normal" control in this experimental model of OA. This contention also is supported by a recent report that synovium from the contralateral knee joint synthesizes hyaluronan at an increased rate (Manicourt et al. in press).

Most, but not all, studies of serum KS in patients with OA have reported that the level was elevated (see Thonar et al. 1993 for review). The serum level of agKS in patients with OA correlates positively with the number of joints involved, the presence of Heberden nodes, subchondral bone sclerosis and a propensity to form osteophytes (Sweet et al. 1988, Campion et al. 1991). As in the case of knee joint injury, the rise in the serum level of agKS probably develops during the preradiological stages of the disease. It is worth noting that the serum level does not show a further rise as the disease progresses to involve more joints or as cartilage erosion becomes more severe (Sharif et al. in press). The results are consistent with the view that the rise in the serum level of agKS precedes the development of clinically assessable changes. We have hypothesized that normal adults with an abnormally high serum level of agKS are at an increased chance of developing OA (Thonar et al. 1994). In this regard, it is worth noting that the serum level of agKS undergoes a marked rise in 6–10% of normal controls between the ages of 25 and 35 (Thonar et al. 1993). One may speculate that a significant proportion of these individuals will develop OA.

Metabolism of aggrecan in inflammatory joint diseases

Levels of agKS in synovial fluid (Shimozuru et al. 1995) and serum (Mehraban et al. 1991, Seibel et al. 1991, Thonar et al. 1991) are, on average, lower in patients with rheumatoid arthritis (RA) than in patients with OA. The observation that serum levels in RA patients correlate inversely with the levels of several serum markers of inflammation, but especially tumor necrosis factor alpha (TNF- α), suggests that inflammatory cytokines circulating in the bloodstream can, directly or indirectly, modulate the metabolism of aggrecan (Manicourt et al. 1993). The

finding that high serum levels of TNF- α are associated with low serum levels of agKS is interesting since incubation of cartilage with TNF- α in vitro causes a marked increase in the rate of aggrecan catabolism (Dingle 1991). As TNF- α is a most effective inhibitor of aggrecan synthesis in vitro (Dingle 1991), it is possible that the decrease in the rate of entry of KS-bearing aggrecan fragments into the blood may reflect a decrease in the size of the pool of newly-synthesized aggrecan which is turned over rapidly in the pericellular matrix. This apparent suppression of both anabolic and catabolic processes may have profound consequences for patients with RA since it would hamper attempts by the chondrocytes to repair matrix when the latter is damaged by enzymes produced by the overlying synovial proliferation.

Children with juvenile RA also have serum levels of agKS which are lower than those of age-matched normal controls (Pachman et al. 1987). As aggrecan plays a key role in the elongation of long bones, a cytokine-induced suppression of their synthesis and turnover would help explain why they are growing more slowly than normal children (Pachman et al. 1987). In an attempt to control rampant inflammatory changes, many of these children receive corticosteroids which are potent inhibitors of aggrecan synthesis and turnover. Efforts should therefore be made to develop new anti-inflammatory drugs that can inhibit cytokine production without shutting down the metabolism of the chondrocytes.

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