

Collagen II turnover in joint diseases

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Content and structure in cartilage

Type II collagen is one of the primary structural components of hyaline cartilage, constituting approximately half the dry weight of extracellular matrix. It is composed of a triple helix of three identical α chains organized in fibrils within the extracellular matrix. Whereas these fibrils are ordinarily clearly visible throughout the matrix, close to the cell in the pericellular domain, their identity is often difficult to discern. It is here that there is likely more active turnover of collagen, such as in rheumatoid arthritis (Månsson et al. 1995).

Type II procollagen contains amino and carboxy propeptides which are removed extracellularly by amino and carboxy proteinases. The resultant molecules form fibrils which occupy a pivotal position in the structural hierarchy of the extracellular matrix. A number of molecules directly interact with the fibril or are part of the fibril. Type XI collagen, of which the α_3 (X1) chain is also encoded by the COL-2A gene, is present together with type IX collagen (Mendler et al. 1989; Poole 1993). The proteoglycans decorin and fibromodulin may sometimes also be present (Poole, 1993). In hypertrophic cartilage, type X collagen is also closely associated with collagen fibrils (Poole and Pidoux 1989, Schmidt and Linsenmayer 1990). Hyaluronic acid (hyaluronan) also interacts with the fibril through, as yet, unknown mechanisms, (Poole et al. 1982). Proteoglycan aggrecan is also attached to the fibril (Poole et al. 1982). But whether this is a real or an artificial association is still unclear. Aggrecan mainly binds to hyaluronan in interfibrillar sites (Poole et al. 1982).

The retention of these macromolecular aggregates and other molecules by the collagen fibril is, of course, dependent upon the integrity of the fibril. In human osteoarthritis there is excessive damage to collagen fibrils (Hollander et al. 1994). This first

occurs at and just under the articular surface. There is a loss of decorin, aggrecan and hyaluronan in these sites, as reflected by radioimmunoassay or radioassay (Poole, Ionescu, Reiner, Fisher, Brooks Roughley, in preparation). The 'classical' loss of proteoglycan staining seen in osteoarthritic cartilages at and close to the articular surface in early degeneration is observed precisely where collagen denaturation is detected immunohistochemically (Hollander et al. 1995).

The pivotal importance of the fibril is also demonstrated by recent studies of molecular defects in these collagens which lead to the premature development of a type II based defect in premature familial osteoarthritis (Ala-Kokko et al. 1990, Eyre et al. 1991) or premature articular degeneration in mice with a type IX defect (Nakata et al. 1993).

Development of new immunochemical methods for the study of type II collagen synthesis

Since this molecule is synthesized and secreted as a procollagen we developed an immunoassay based on the C-propeptide so that we could study type II procollagen synthesis (Hinek et al. 1987). By immunoprecipitation of radiolabeled newly synthesized molecules we previously showed that the C-propeptide is rapidly removed following synthesis (Kujawa et al. 1989) and that its half-life ($t_{1/2}$) in bovine and human cartilages is relatively short being about 16–18 hr (Nelson et al. unpublished). Moreover, the content of the C-propeptide directly correlates with the rate of procollagen II synthesis.

Studies of osteoarthritic compared to normal human femoral condylar cartilages have revealed marked increases in the content of the C-propeptide in osteoarthritis (Nelson et al. unpublished). This confirms other work revealing increased type II collagen synthesis in the human disease (Aigner et al.

1993). Analyses of the distribution of the C-propeptide by immunohistochemistry and immunoassay have revealed that most of this increased synthesis occurs in the mid and deep zones of articular cartilage in osteoarthritis, whereas in normal cartilage, the C-propeptide usually seems to be evenly distributed throughout the tissue (Nelson et al. unpublished). In osteoarthritic cartilages, analyses of the relative contents of the C-propeptide and a new epitopic marker of aggrecan synthesis, designated as the 846 epitope (Rizkalla et al. 1992, Jugessur and Poole unpublished) have revealed a moderately significant correlation, suggesting that both collagen and aggrecan synthesis are altered in osteoarthritis, as suggested originally (Lippiello et al. 1977, Mankin and Lippiello 1970).

We have recently investigated the response of articular cartilage to acute inflammation initiated by intra-articular injection of ovalbumin into rabbits presensitized to this molecule (Lundberg et al. unpublished). This produces an acute, then chronic erosive inflammatory arthritis. The damage to articular cartilage is characterized by a loss of proteoglycan and a more rapid transient early increase in the content of the C-propeptide reflecting enhanced procollagen II synthesis. This occurs when aggrecan synthesis is inhibited as reflected by a decrease in the 846 epitope.

The C-propeptide is also detectable in body fluids where its presence may reflect either local or systemic changes. Joint fluid analyses in rheumatoid and osteoarthritis have revealed its presence in synovial fluid (Poole, Ionescu, Swan and Dieppe, unpublished). But, although concentrations of this propeptide are not generally very elevated over serum levels, they may reflect local cartilage changes. We have noted inverse changes in levels of the C-propeptide and levels of keratan sulfate, a marker of aggrecan degradation (Poole, Ionescu, Swan and Dieppe, unpublished). This suggests that there may exist recognizable phases of net cartilage synthesis (repair) or degradation (pathological damage).

In early rheumatoid arthritis it is not uncommon to observe elevations in levels of the C-propeptide in serum even in cases of severe inflammatory disease with rapid erosive joint destruction (Månsson et al. 1995). This would appear to reflect a compensatory increase in collagen II synthesis under conditions where collagen II degradation is almost certainly enhanced. Such elevations are also observed, albeit to a smaller degree, in sera of about 20% of patients with osteoarthritis (Poole et al. 1994). Interestingly, we have found that there is no evidence for increased aggrecan synthesis (846 epitope) in these patients

with early destructive rheumatoid arthritis. This may reflect selective impairment of aggrecan synthesis by cytokines. These studies are still very much in their infancy and further work is required to establish the value of this C-propeptide in *in vivo* analyses of cartilage metabolism.

Degradation

Collagen α chain cleavage results in denaturation of the triple helix if this occurs within the helical region. The only known enzymes capable of cleaving triple helical type II collagen are interstitial collagenase (MMP-1, or collagenase-1) neutrophil collagenase (MMP-8, or collagenase-2), and the recently discovered collagenase-3 (MMP-13). Any of these proteinases can cause denaturation of the triple helix whereas proteinases that cleave in non-helical telopeptide domains, such as stromelysin, cannot disrupt the integrity of the triple helix, although they can result in loss of fibril function (Wu et al. 1991).

Previously, it was not possible to detect collagen cleavage *in situ* other than with morphological methods which were used to recognize fibril damage. We succeeded in developing an immunochemical method to detect helix denaturation by developing and using antibodies that react only with an intra- α -chain epitope(s) in the helical region of the denatured molecule. These antibodies show no reactivity with triple helical (native) type II collagen (Dodge and Poole, 1989, Hollander et al. 1994). By using α -chymotrypsin to digest denatured collagen and proteinase K to solubilize all collagen without cleaving the epitope recognized by a monoclonal antibody (COL2-3/4m), we were able to establish an ELISA assay to determine total and denatured collagen content (Hollander et al. 1994). Moreover, we could use the antibodies in immunolocalization studies to detect sites of collagen denaturation within the tissue (Hollander et al. 1995).

These studies have extended earlier biomechanical analyses of osteoarthritic cartilages that indicated a loss of tensile properties in arthritis and hence increased collagen fibril damage (Kempson et al. 1973). We found that collagen denaturation was increased in adult femoral condylar cartilages usually from a range of 0-2% to 2-9% (Hollander et al. 1994). This level of enhanced denaturation is significantly higher than in normal adult cartilage but actually compares to that found in fetal and newborn articular cartilages (Hollander and Poole, unpublished). In fact, in the bovine growth plate hypertrophic zone, up to 60% of the collagen can be denatured. It is thus an interesting observation that the level of damage to collagen in disease is no more than is found in comparable sites in healthy rapidly grow-

ing cartilage. What is different is the level of synthesis, based on C-propeptide analyses in osteoarthritis: this is less than 25% of that observed in the fetus or newborn. Thus, the pathology is characterized by excessive degradation with a lack of a sufficient compensatory increase in synthesis. The fate of these newly synthesized molecules is also unclear.

Which fibrils are being excessively degraded in growth versus arthritis is, also, unclear. We know that the large fibrils that ordinarily are destined to survive for the life of the individual, once the growth plates have closed, are damaged in arthritis (Dodge et al. 1991). But it is possible that newly synthesized molecules may also be excessively damaged in arthritis. Interestingly we often find, by immunohistochemistry, denaturation of type II collagen in the same pericellular and territorial sites where the staining for the C-propeptide is increased. This is seen in both rheumatoid arthritis and osteoarthritis. We need to understand more about these processes so we can try and maximize synthesis over degradation and restore the balance. What was surprising in comparative analyses of collagen II and aggrecan synthesis was that C-propeptide levels in osteoarthritic cartilages are increased over normal to ~25% of normal levels at birth.

The increase in synthesis of type II procollagen is also observed physiologically in association with enhanced collagenolysis. In the hypertrophic region of the growth plate, synthesis increases as cells become hypertrophic and synthesize type II procollagen (Sandberg and Vuorio 1987, Leboy et al. 1988). This corresponds to increased denaturation of type II collagen both in situ (Alini et al. 1992) and in vitro (Alini et al. 1994). There is, however, a net loss of collagen II associated with the resorption of the extracellular matrix as the cells enlarge in size. But this is a well regulated physiological process.

In arthritis, collagen II denaturation is clearly recognizable in specific sites which provide insights into its pathogenesis. In ageing and osteoarthritis this is always first detected at and just under the articular surface from which it extends progressively deeper with increasing Mankin grade (Hollander et al. 1995). At this stage this is not accompanied by detectable increases in the C-propeptide of this molecule.

The first collagen II denaturation is always detected immediately at the edge of the chondrocyte indicating that the degradation is mediated by this cell. This is what we observe when we stimulate chondrocytes in culture with interleukin-1 (Dodge and Poole 1989, Mort et al. 1993). The progressive nature of this disease may result from changes in biomechanical forces acting upon the cell resulting from

neighboring damage to the matrix. Altered loading is known to have profound effects on cartilage matrix turnover (Poole 1993). The prodegradative cytokines such as interleukin-1 and tumor necrosis factor- α , may also be involved.

Fibrillation is ordinarily only observed once the damage to the matrix has extended at least 2/3 the way to the subchondral bone (Hollander et al. 1995). Comparative immunohistological analysis of normal and osteoarthritic cartilages suggest very similar pathology involving collagen II denaturation in ageing after 30–40 years. But quantitative analyses reveal that the main difference is one of the amount of collagen II denaturation, this being markedly increased in arthritis. Wherever we observe cell cloning, we note excessive damage and increased synthesis of type II collagen. In some sites, less damage is observed in territorial sites suggesting that reparative processes may be partly effective.

These changes contrast to rheumatoid arthritis where we detect damage throughout the matrix in advanced disease and to the same degree as in osteoarthritis by immunoassay. But there is no evidence of fibrillation. Coupled with this we never see evidence of excessive damage in more superficial sites in rheumatoid cartilage as in osteoarthritis where fibrillation starts in association with the greatest damage. In early rheumatoid disease, damage can also be pronounced around chondrocytes in the deep zone adjacent to subchondral bone. This points to local activation of chondrocytes by prodegradative cytokines originating from bone (Dodge and Poole, 1989). At the junction with pannus, collagen denaturation is always very pronounced: but so is procollagen synthesis.

Our aim now is to be able to identify the mechanisms responsible for increased matrix degradation in arthritis, both at the enzymatic and cellular levels, so that regulation of synthesis and the activities of proteinases can be controlled. Can we enhance synthesis and protect newly synthesized molecules as well as inhibiting matrix degradation? This now looks feasible.

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