Binding sites for CMP were more abundant and the extent of decoration with CMP increased with aging.

It appears that several pools of CMP are found in the cartilage matrix. These may contribute different properties and functions. One pool represents tightly bound CMP that can not be released by detergents, chaotropic agents nor by reduction. Upon maturation and aging of the tissue a marked increase in total CMP-content is accompanied by a characteristic shift from unbound to tightly bound CMP.

References

Hauser N, Paulsson M. Native cartilage matrix protein (CMP)—a compact trimer of subunits assembled via a coiled-coil α-helix. J Biol Chem 1994; 269: 25747–53.

Paulsson M, Heinegård D. Radioimmunoassay of the 148-kilodalton cartilage protein—distribution of the protein among bovine tissues. Biochem J 1982; 207: 207–13.

Distribution of CMP and COMP in human cartilage

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Cartilage matrix protein (CMP) is a noncollagenous glycoprotein with a molecular mass of 148 kDa consisting of three identical subunits linked together at their C-terminal assembly domains via a coiled-coil α-helix (Hauser and Paulsson 1994). Electron microscopy of the native protein showed trimers with compact ellipsoid subunits, each consisting of two von Willebrand factor A-domains linked via one EGF-like repeat. CMP is a highly insoluble protein which appears to become covalently crosslinked within the cartilage matrix with maturation.

Cartilage oligomeric matrix protein (COMP) has been purified in a native form from articular cartilage (DiCesare et al. 1994a, 1995) and is a pentameric protein consisting of identical subunits with a molecular mass of 110 kDa. COMP belongs to the thrombospondin family and consists of a C-terminal globular domain, followed by seven type III repeats, which have been implicated in calcium binding, four EGF-like repeats and finally an N-terminal assembly domain forming a pentameric coiled-coil α-helix under native conditions. COMP is ubiquitously distributed throughout all different kinds of cartilage and is also found in non-cartilagenous tissues as e.g. tendon (DiCesare et al. 1994b). COMP has been shown to bind chondrocytes (DiCesare et al. 1994a) and could play a role in contacts between extracellular matrix and chondrocytes.

Both COMP and CMP have been used as markers for cartilage degradation in human samples (Lohmander 1994), but studies of their tissue distribution have mainly been done in steer. We therefore undertook to determine their distribution and concentration in human cartilages.

Material and methods

Tissues were obtained in the Department of Forensic Medicine, University of Bern, from recently deceased (within 20 hours) accident or murder victims and used directly.

Extraction of tissues was performed with 4 M guanidine HCl (10 ml per gram of wet tissue) for 16 hours at 4°C after brief homogenization with a Polytron homogenizer.

Polyclonal antisera against bovine CMP and against human COMP, were raised in rabbits.

For immunohistochemistry, sections (5 μm) were cut, digested for 1 h with 40 mU/ml chondroitinase ABC in TBS containing 0.01% (wt./vol.) bovine serum albumin (BSA), incubated in methanol containing 1% (vol./vol.) H_2O_2 and in 1% (wt./vol.) BSA in TBS. Sections were treated with the specific antiserum or nonimmune rabbit serum for 1 hour, followed by peroxidase-conjugated swine anti-rabbit IgG for 45 min. The slides were developed with 0.025% (wt./vol.) 3-amino-9-ethylcarbazole/0.02% (vol./vol.) H_2O_2.

Inhibition ELISA was performed in microtiter plates coated overnight at 4 °C with antigens at 5 mg/ml in TBS and blocked with 1% BSA in TBS for 2 hours at 22–24 °C. Serial dilutions of the extracts were preincubated with the corresponding antibodies, added to the coated plates, and binding of antibodies detected using a secondary antibody to rabbit IgG conjugated with peroxidase and 5-amino-2-hydroxy benzoic acid as a substrate.

Results and Discussion

To determine CMP and COMP in human tissues, we examined extracts of articular cartilage from tibia and femur, tracheal cartilage and rib cartilage, anulus fibrosus and nucleus pulposus, meniscus and cruciate ligament from a 45-year-old man.

For the determination of COMP, native human COMP was purified from articular cartilage, using the protocol of DiCesare et al. (1995). Purification of native human CMP was hampered by the very low solubility of this protein. Therefore native CMP purified from fetal bovine rib cartilage (Hauser and Paulsson, 1994) was used. Polyclonal antibodies against native human articular COMP and native bovine rib CMP were raised in rabbits. Specificity and crossreactivity were verified on immunoblots of crude cartilage extracts.

The highest levels of COMP, measured by ELISA, were in the meniscus, 3.47 mg/g tissue (wet weight), and in articular cartilage of the tibial plateau, 3.40 mg/g. The lowest concentrations were found in the rib and the trachea with 0.26 and 0.04 mg/g, respectively. The results obtained by ELISA were confirmed qualitatively by immunoblots.

In immunoblots the staining pattern for CMP is very clear; a single band at 50 kDa was obtained after reduction for the tracheal and the rib extract, while all other extracts were negative. Thus, human CMP from tracheal and rib cartilage has the same electrophoretic mobility as the corresponding protein purified from bovine rib cartilage. COMP was detected as a band with the same mobility as the purified standard throughout the extracts, with a gradient in intensity agreeing with the amounts determined by ELISA.

The findings were confirmed by immunohistochemistry of cryosections of the same specimens. The expression of CMP is limited to tracheal and rib cartilage, where a particularly strong staining is seen in the perichondrium. Antibodies to COMP stained homogeneously throughout all types of tissues examined.

We have shown that CMP occurs only in tracheal and rib cartilage in the human adult and not in the other tissues examined. The inhibition ELISA to determine human CMP involved the use of native protein purified from fetal bovine rib cartilage and antibodies against bovine CMP. Quantitative results could not be obtained, presumably because of the lack of human reagents. Measurement of COMP involved the use of native protein purified from human articular cartilage and polyclonal antibodies raised against human COMP and accordingly truly quantitative measurements could be obtained.

The changes of tissue distribution and quantities of CMP and COMP with aging of the individuals has not yet been comprehensively analyzed. A longitudinal study of CMP in bovine tracheal cartilage showed dramatic increases in amounts with aging (Paulsson and Heinegård, 1982). Similar work needs to be done in the human system and could yield information relevant to the function of these two proteins.

IL-1 exerts diverse effects on the metabolism of articular chondrocytes, including inhibition of proteoglycan synthesis and stimulation of matrix metalloproteinase synthesis. Therefore it is believed that IL-1 might play an important role in cartilage degradation in osteo- and rheumatoid arthritis. To improve our understanding of IL-1 induced effects on overall gene expression patterns of human articular chondrocytes, we used a novel mRNA fingerprinting technique: Differential Display Reverse Transcription–PCR (DDRT-PCR) (Liang and Pardee 1992). The reported high sensitivity of this powerful technique promised to enable work with human articular cartilage, a tissue from which only small amounts of RNA can be obtained, and to get access to low abundance transcripts, difficult to detect with conventional subtractive hybridization studies. Our goal was to eventually identify new marker genes and pharmacological target molecules for drug intervention in IL-1 mediated cartilage degradation.

References

DiCesare P E, Hauser N, Lehman D, Pasumarti S, Paulsson M. Cartilage oligomeric matrix protein (COMP) is an abundant component of tendon. FEBS Letters 1994; 354: 237–40.

DiCesare P E, Mörgelin M, Mann K, Paulsson M. Cartilage oligomeric matrix protein and thrombospondin 1, purification from articular cartilage, electron microscopic structure, and chondrocyte binding. Eur J Biochem 1994; 223: 927–37.

DiCesare P E, Mörgelin M, Carlson C S, Pasumarti S, Paulsson M. Cartilage oligomeric matrix protein (COMP): isolation and characterization from human articular cartilage. J Orthop Res 1995; in press.

Hauser N, Paulsson M. Native cartilage matrix protein (CMP)—a compact trimer of subunits assembled via a coiled-coil α-helix. J Biol Chem 1994; 269: 25747–53.

Lohmander L S. Articular cartilage and osteoarthrosis. The role of molecular markers to monitor breakdown, repair and disease. J Anat 1994; 184: 477–92.

Paulsson M, Heinegård D. Radioimmunoassay of the 148-kilodalton cartilage protein—distribution of the protein among bovine tissues. Biochem J 1982; 207: 207–13.