Synthesis of abnormal articular cartilage proteoglycans in rapidly destructive arthropathy (osteoarthritis)

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Received July 16, 1990/Accepted January 18, 1991

Summary. Articular cartilage fragments were obtained from four femoral heads and one femoral condyle, resected in five patients undergoing prosthetic surgery for rapidly destructive arthropathy (RDA) and from one normal femoral head and one normal femoral condyle resected at autopsy. The cartilage fragments were labelled in vitro with $^{35}$S and newly-synthesized proteoglycans ($^{35}$S-PGs) were then extracted with 4 M guanidine hydrochloride (GuHCI) and analyzed. In three cases a much greater and in one case a significantly increased proportion of small $^{35}$S-PGs enriched in dermatan sulfate (DS) was demonstrated in diseased tissues in comparison with control samples. These DS $^{35}$S-PGs were completely unable to interact with hyaluronan (HA) and had longer glycosamino-glycan (GAG) side chains than large $^{35}$S-PGs. Also, large $^{35}$S-PGs extracted from diseased tissue interacted poorly under associative conditions with exogenous HA when this was added to the crude extract. However, they interacted much better following the addition of exogenous HA to the purified high density proteoglycans. This suggests the presence of an inhibitor of PG-HA interaction in the crude extract which is lost during PG purification. The synthesis of an abnormally large proportion of small PGs by articular chondrocytes and impaired aggregation of large PGs may account for the accelerated destruction of articular cartilage in this condition.

Key words: Destructive osteoarthritis – Articular cartilage – Synthesis of abnormal proteoglycans

Introduction

Rapidly destructive arthropathy (RDA) is a peculiar form of osteoarthritis (OA) characterized by the rapid disappearance of articular cartilage and destructive-reconstructive subchondral bone changes [1, 2]. This disorder mainly affects women aged over 50 years and apparently develops in the absence of any known etiological factor. The hip, knee and shoulder are the joints most often involved. This condition may develop in normal joints or complicate the course of common OA.

The mechanism of cartilage destruction in this disorder is unknown. Various factors such as crystals, therapeutic drug abuse, obesity, and overuse of joints [1, 2] have been suspected but their role has not received general acceptance. More recently, B2-microglobulin synovial amyloidosis was found to be consistently associated with RDA of hemodialysis [3], though its role in joint destruction is doubtful.

Proteoglycans (PGs) and collagen are two of the main components of the articular cartilage extracellular matrix. Articular cartilage PG is a composite molecule consisting of a core protein to which are covalently attached variable numbers of chondroitin sulfate (CS), keratan sulfate (KS), O-linked and a few N-linked oligosaccharide chains [4–6]. In the matrix of normal articular cartilage, an overwhelming proportion of PGs are of large type. Most of them interact with other components such as hyaluronan (HA) and glycoproteins, and form large molecular structures or aggregates. A small proportion of PGs are of small type [7]. They contain one or two CS chains [8] with some glucuronate residues, having been epimerized to iduronate. Two distinct dermatan sulfate PGs have been described in bovine [9] and human [10, 11] articular cartilages. They represent distinct gene products and are found in other connective tissues as major components [12, 13]. In recent years articular cartilage type IX collagen has been shown to bear a CS chain and to be covalently crossed linked to type II collagen [14].

Because of their high negative charge, PGs are highly hydrophilic and form a gel that is immobilized in a three-dimensional collagen fibrillar network. This hyperhydrated gel gives the tissue its stiffness and elasticity and enables the cartilage to resist and distribute compressive forces to the subchondral bone [15]. Changes in PG content and quality are an early event in OA [16–18], and are thought to predispose articular cartilage to superficial fibrillation and progressive abrasion of the tissue as a result of mechanical shear forces.
In another paper (manuscript in preparation), we will report the macroscopic and histological findings concerning the synovium, articular cartilage and subchondral bone of 11 femoral heads resected surgically in nine patients suffering from RDA of the hip. In this paper, we examined the structure, chemical composition and aggregating properties of newly-synthesized PGs extracted from articular cartilage removed from resected femoral heads of five patients and compared these with corresponding molecules extracted from femoral head articular cartilage.

Material and methods

Normal femoral head and femoral condyles were obtained at autopsy from a fresh (12 h after death) cadaver of a male subject who died at the age of 55 years from a heart attack. The diseased material was obtained at surgery from five patients operated at stage 4 of Kellgren's scale [19].

Brief patient histories and description of resected material

Patient 1. This 57-year-old female suffered from bilateral hip dysplasia and secondary OA of both hips. The clinical symptoms associated with the right hip suddenly worsened and two consecutive X-rays taken under load at a 3-month interval showed that more than 50% of the hip joint space was lost. The patient was operated on 3 months later when at least 90% of the joint space had disappeared. Synovial fluid was not obtained at surgery. The resected femoral head exhibited typical OA changes with a large zone of exposed bone on the superior aspect and small osteophytes at the periphery. The synovium was slightly hypertrophic but not inflammatory. Articular cartilage around the ulceration was fissured and normal cartilage was present mainly on the inferior aspect of the femoral head. Grossly normal and fissured cartilage as well as surface covering from peripheral osteophytes were dissected for analysis.

Patient 2. This 75-year-old female also suffered from bilateral hip dysplasia with secondary OA which was more advanced in the left hip. She also experienced accelerated destruction of the left hip joint space which was complete in about 6 months. There was also significant upward displacement of the femoral head. Synovial fluid was not obtained at surgery but the synovium was hypertrophic and granular. The resected femoral head was considerably flattened and exhibited a large ulceration on its superior aspect and prominent osteophytes at the periphery. The articular cartilage remaining around the ulceration was thinned, softened and mainly fissured. Primitive, grossly normal or fissured hyaline articular cartilage and surface covering from peripheral osteophytes were dissected for analysis.

Patient 3. This 68-year-old female presented with bilateral genu valgum and secondary OA affecting particularly her left knee in both femoro-patellar and femoro-tibial compartments. She experienced pain and walking difficulties. X-rays taken under load indicated a 75% reduction of the external femoro-tibial joint space over 9 months. Synovial fluid was not obtained at surgery, and the synovium was slightly hypertrophic but not inflammatory. Resected femoral condyles and tibial plateaue exhibited large ulcerations revealing erburnated bone and numerous small peripheral osteophytes. A rim of thinned and softened grossly normal hyaline cartilage lying around the ulcerated area and surface covering from peripheral osteophytes were dissected for analysis.

Patient 4. This 54-year-old female suffered from pain and walking difficulties associated with her left hip. The first X-ray was normal, though 6 months later, an X-ray taken under load showed a 70% loss of joint space. She was operated on 2 months later, when 2–3 ml of discoloured brown synovial fluid was withdrawn, by transcapsular aspiration. The resected femoral head was flattened and the articular cartilage was considerably thinned, softened and fissured all over the articular surface, particularly on the superior aspect of the femoral head. Thinned and fibrillated cartilage from the superior aspect was dissected for analysis.

Patient 5. This 72-year-old male suffered from pain in the right hip. A diagnosis of OA was established on X-ray and the patient was seen 6 months alter. The patient's status had worsened and a 30% loss of joint space was observed on the new X-ray. Three months later the X-ray revealed an additional 60% loss of the joint space and upward displacement of the femoral head. The patient was operated on 2 years after the first examination. At operation, 2–3 ml of citrous and viscous synovial fluid were withdrawn by transcapsular aspiration. Grossly, the synovium was hypertrophic and granular. The resected femoral head was flattened. It’s superior aspect was ulcerated and spotted with plugs of fibre-cartilage. A thin rim of normal hyaline cartilage persisted around the ulcerated area. Small immature osteophytes were present, in particular at the periphery of the inferior aspect of the head. Grossly normal cartilage surrounding the ulcerated area, tissue from the ulcerated area and surface covering from peripheral osteophytes were dissected for analysis.

All patients were treated with the usual doses of analgesics and non-steroidal anti-inflammatory drugs and were given 5–10 mg of prednisone per day for 2 days before and on the day of surgery.

Processing of resected material. The resected material was immediately placed in cold Ham's F-12 culture medium and brought on ice to the laboratory. Primitive, grossly normal and fibrillated articular cartilage remaining on the femoral heads and femoral condyles, soft tissue found on ulcerated articular surfaces and surface covering from peripheral osteophytes were dissected under sterile conditions and pooled separately for each case in sterile Falcon 9-cm plastic Petri dishes (Becton, Dickinson and Co, Grenoble, France) containing 20 ml of Ham’s F-12 culture medium and antibiotics (penicillin 100 IU/ml and streptomycin 50 µg/ml). The water binding capacity of cartilage fragments was determined on aliquots of representative samples by weighing wet and corresponding freeze-dried aliquots.

Labeling of tissue fragments. The remaining fragments were then transferred to the same medium adjusted to 25 µCi/ml of [35S]Na2SO4 (spec. act. 460.2 mCi/mmol, New England Nuclear, Du Pont de Nemours Co, Paris, France). Cultures were placed in a CO2 (5%) incubator at 37°C for 24 h. Incorporation was stopped by withdrawing the radioactive medium and rinsing the tissue fragments with phosphate-buffered saline (PBS) buffer containing protease inhibitors (10 mM N-ethylmaleimide, 10 mM ethylenediaminetetraacetic acid (EDTA), 10 mM l-aminocaproic acid, 1 mM benzamidine, 1 mM O-phenanthroline and 0.5 mM phenylmethylsulfonyl fluoride).

Rinsed tissue fragments were finely sliced with a razor blade and then carefully hand-squeezed in a porcelain mortar. The crushed cartilage fragments were recovered by rinsing the mortar with distilled water. The materials from each case and tissue were freeze-dried and weighed on a precision Mettler balance.

Extraction of PGs. One gram of dry tissue was extracted with two volumes, each of 10 ml of 0.05 M Tris HCl buffer, pH 7, containing 4 M guanidinium hydrochloride (GuHCl) and protease inhibitors as above (dissociative buffer). Each extraction step was carried out at 4°C, for 24 h with constant gentle stirring. The extract was separated from the residue by centrifugation for 1 h at 12 100 g and at 4°C. Extracts 1 and 2 were pooled, concentrated at 4°C through an Diaflo YM 10 filter membrane (10 000 MW cut off, Amicon Co, Danvers, MA, USA) to one-tenth of their initial volume and adjusted similarly to associative conditions with the above buffer without GuHCl. This step usually took 4–5 h. The extracts were aliquoted and stored at −80°C until analyzed. Extracted residues were rinsed with distilled water, freeze-dried and weighed, as above.