Osteoarthritis and Cartilage 24 (2016) 1029–1035

Osteoarthritis and Cartilage

Evidence for enhanced collagen type III deposition focally in the territorial matrix of osteoarthritic hip articular cartilage

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ARTICLE INFO

Article history:
Received 2 October 2015
Accepted 1 January 2016

Keywords:
Hip osteoarthritis
Articular cartilage
Collagen type II and type III

SUMMARY

Objective: To determine if type III collagen is concentrated in the chymotrypsin-extractable collagen pool from osteoarthritic articular cartilage to assess its potential as a biomarker of Osteoarthritis (OA) pathogenic mechanisms.
Methods: Full thickness articular cartilage from grossly normal surfaces was analyzed from femoral heads, obtained at hip replacement surgery, from OA ($n = 10$) and fracture ($n = 10$) patients. Collagen, extracted by α-chymotrypsin, was characterized by SDS-PAGE/Western blot analysis, ELISA and immunohistochemistry using monoclonal antibodies specific to collagens types II and III.
Results: α-Chymotrypsin extracted more collagen from OA than control cartilage. The extractable pool included collagen types II and III from both OA and control hips. Importantly, OA cartilage contained 6-fold more collagen type III than control cartilage, based on ELISA. The estimated total tissue ratio of collagen III/II was in the 1–10% range for individual OA cartilage samples, based on pepsin-solubilized collagen using SDS-PAGE densitometry. Collagen type III N-propeptide trimers were the main molecular fragments seen on Western blot analysis of OA and control extracts. The chymotrypsin-extracted type II collagen gave primarily full-length α1(II) chains and chain fragments of α1(II) on Western blot analysis from both OA and control tissues. Immunohistochemistry showed that type III collagen was more concentrated in the upper half of OA cartilage and in the territorial matrix around individual chondrocytes and chondrocyte clusters.
Conclusions: The findings confirm that collagen type III deposition occurs in adult articular cartilage but significantly more pronounced in osteoarthritic joints, presenting a potential marker of matrix repair or pathobiology.

Introduction

Osteoarthritis (OA) is a multifactorial disease involving the whole joint in which loss of cartilaginous matrix is a fundamental feature. Chondrocytes have great potential to replace the proteoglycan components of the matrix. However, in the late stages of OA when collagen damage is far advanced, any repair attempt appears to be ineffective leading eventually to joint failure.

The collagen network of mature human articular cartilage develops from a heteropolymeric microfibril network of type II collagen polymerized on and covalently bound to a type XI collagen template and to type IX collagen molecules, which coat initial fibril surfaces[1]. Once laid down during development, there is little evidence that articular chondrocytes can recapitulate the overall collagen network architecture if the mature tissue is damaged by injury or degeneration. Collagen turnover in mature articular cartilage is very slow. A turnover time of 400 years for collagen of human femoral head cartilage was estimated from the synthetic rate of hydroxyproline[2]. However, some remodeling can occur[3]. It is possible that the chondrocytes can remodel micro-anatomical domains of collagenous matrix (e.g., replacing fibril-surface molecules, damaged fibrils or pericellular collagen) more rapidly than bulk collagen of the inter-territorial matrix[1].

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http://dx.doi.org/10.1016/j.joca.2016.01.001
1063-4584/

Several studies have shown increased synthesis of collagen in surgically induced OA in animals and in human OA cartilage[4–6]. Type II collagen was the major new product on radiolabeling *in vivo* and, though the data ruled out type I collagen[6], left open the possibility of other collagen types being expressed including type III collagen. Since then, direct evidence has been provided for the appearance of type III collagen in the matrix of adult articular cartilage[7].

Molecular analysis of the pool of extractable collagen showed the presence of collagen type III covalently linked to collagen type II in the matrix of human knee OA cartilage[8]. The findings indicated that pN-type III molecules were self-polymerized and covalently cross-linked to the surface of type II collagen fibrils in the extracellular matrix. This would be consistent with the concept that retained N-propeptides on the surface of procollagen prevents lateral growth of fibrils in the process of assembly[9,10]. Transmission electron-microscopy, using immunogold, showed type III collagen on the surface of banded type II collagen fibrils in human articular cartilage[11]. Low but increasing amounts of type III collagen were also detected in normal adult and OA human articular cartilage, where it was concentrated around chondrocytes throughout the depth[7] or in the surface and upper mid-zones of OA cartilage[12]. Based on mRNA analysis, the expression of collagen type III was associated with expression of collagen type II but not collagen type I in OA cartilage[12]. Together these various findings indicate a metabolic response of chondrocytes to deposit collagen type III in regions of articular cartilage, presumably as a response to mechanical injury or other matrix damage. The effect may be akin to the wound-healing role of collagen type III in skin and other collagen type I-based connective tissues.

A previous study has shown that α-chymotrypsin digestion extracts more collagen from cartilage of OA than control joints[13]. α-Chymotrypsin is believed not to attack the native triple-helical domain of types I and II collagen molecules below the denaturation temperature of the triple-helix. Based on the immunochemical detection of type II collagen breakdown products in such extracts, it was concluded that chymotrypsin extracts a denatured pool of type II collagen, which may already be proteolytically cleaved[14]. However, it is known that native collagen type III, unlike collagen types I and II, is susceptible to cleavage by trypsin, and potentially chymotrypsin, in the domain of labile triple-helix which contains the site where tissue collagenase cleaves[15]. Chymotrypsin is also a candidate telopeptidase, so it could, in theory, depolymerize and solubilize native type II collagen molecules by cross-link breaking cleavages in telopeptide domains.

In a study of cartilage from osteoarthritic femoral heads, more than twice as much collagen was extracted by chymotrypsin than from non-osteoarthritic femoral heads[13]. The molecular nature of this extractable collagen has not been characterized. Therefore, the present study was designed to examine the possibility that collagen type III was prominent in it, to determine the size of the molecular fragments and to explore the potential for insights in OA pathogenesis and the potential for a novel biomarker of the OA process. The availability of well-characterized sets of femoral heads from clinical OA and osteoporotic fracture patients undergoing hip replacement surgery made this collaborative study possible.

Methods

Patient tissue source

Femoral heads (10 OA and 10 controls) were obtained at total hip replacement surgery from either patients with OA or femoral neck fracture (i.e., controls). After surgery all femoral heads were kept frozen at −80°C. The femoral neck fracture patients were mostly elderly females and had typical osteoporotic neck fractures. Cases of known RA and secondary OA were excluded from the study. The cartilage of OA samples, obtained from the OA patients at total hip replacement surgery, had Kellgren and Lawrence grade 2 or more.

Cartilage samples from five OA (patient age-range 60–80 yr, four female) and five control femoral heads (patient age-range 78–87 yr, five female) were analyzed for total collagen content and chymotrypsin-extractable collagen (Experiment 1) (Table I). Cartilage samples from another five OA (patient age-range 50–80 yr, four female) and five control heads (patient age-range 53–87 yr, two female) were used to study pepsin-extracted collagen (Experiment 2) (Table II). The sampled area of cartilage

Table I
Yields of chymotrypsin-extractable collagen from OA and control cartilage

	OA ($n = 5$)	Non-OA ($n = 5$)	*P*-value*	Difference OA & Non-OA	Age adjusted *P*-value	Age adjusted-estimate of difference
Age (year)	69 (66.7–74.7)	81 (79.5–85.5)	0.024	−12 (−1 to −21)		
Total hydroxyproline (μg/mg wet wt. cartilage)	23.8 (14.5–24.3)	23.6 (21.5–28.2)	0.548	−4.04 (−13.26–6.78)	0.66	−2.06 (−12.7–8.6)
% Extracted hydroxyproline	0.7 (0.6–0.9)	0.2 (0.2–0.4)	0.008	0.45 (0.18–0.83)	0.097†	0.28 (−0.0066–0.57)
Relative type III collagen content of extracts by ELISA (μg human collagen type III enriched standard/100 mg cartilage)	81 (51.3–134.8)	14.7 (10.1–19.5)	0.008	73.4 (30.2–130)	0.005†	80.7 (42.2–119)

Values are medians (25–75 percentiles).
* Unadjusted; using exact Wilcoxon–Mann–Whitney test.
† Weighted model.

Table II
Proportion of collagen III in OA and control cartilage

	OA ($n = 5$)	Non-OA ($n = 5$)	*P*-value	Difference OA & Non-OA	Age-adjusted *P*-value	Age-adjusted difference
Age (year)	70 (62–79.2)	80 (72.5–85.5)	0.198	−8 (−30–17)		
% Collagen type III (III/II + III in pepsin extract)	5 (0.8–8.1)	0†	0.025	5 (0–9.1)	0.049	3.8 (0.025–7.6)*

Values are medians (25–75 percentiles).
NOTE: Difference is the Lehmann–Hodges estimator of the shift in medians; whereas the adjusted difference is difference in means adjusted for baseline. Both differences are with 95% confidence intervals in both Tables I and II.
* Adjusted for age by group.
† No detectable band with NIH ImageJ software.