Acta orthop. scand. 54, 613–619, 1983

MINOR COLLAGENS IN ARTHROTIC HUMAN CARTILAGE

Change in Content of 1α, 2α, 3α and M-collagen with Age and in Osteoarthrosis

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The content and distribution of 1α, 2α, 3α and M-collagens in human articular cartilage were studied. As controls, normal femoral heads and costal cartilage of autopsy material from newborn to 91-year-old persons were used. The osteoarthrotic cartilage was obtained from patients undergoing total hip replacement aged 45–80. The pepsin-digested cartilage collagen was fractionated by differential salt fractionation. The collagen content of the fractions was determined, and the fractions were separated by polyacrylamide slab gel electrophoresis. In the extracted collagen, the type II collagen varied from 82 to 97 per cent with increasing age. The 1α, 2α and 3α chains decreased. M-collagen, especially of the high molecular weight components, disappeared with age.

In ostearthrosis three types of change – degeneration, new fibrocartilage formation on the surface of osteophyte and reparative cartilage – were separately studied. In all types of osteoarthrosis, an increase of minor collagens was found. In newly formed fibrocartilage, the reappearance of M-collagen was conspicuous. It is proposed that the three types of osteoarthrotic cartilage may be characterized on the basis of content and distribution of minor collagens.

Key words: aging; cartilage, articular; collagen; femur head; osteochondritis

Accepted 9.ii.83

Five distinguishable collagen chains in addition to and different from cartillage specific collagen type II were described in human femoral and costal cartilage by Burgeson & Hollister (1979), in pig hyaline cartilage by Shimokomaki et al. (1980) and in bovine nasal cartilage by Ayad et al. (1981). These new collagen chains were verified and characterized by Reese & Mayne (1981), Reese et al. (1981) and von der Mark et al. (1982) in costosternal chicken cartilage as well. Compared to interstitial collagens these chains have higher solubility in dilute acid, and different content of alanine and hydroxylysine. They are similar to but distinguishable from collagen type V. These chains, named minor collagens by Reese & Mayne (1981), are 1α, 2α, 3α, the last presumed to be a glycosylated variant of collagen type II, and M-collagens. The high and a low molecular weight chains were named HMW and LMW M-collagens by Reese & Mayne (1981), and M_1 and M_2 by von der Mark et al. (1982). Shimokomaki et al. (1980) recommended the term M-collagen, whereas Ayad et al. (1981) proposed C-PS (phosphate soluble collagen) for the same collagen component. The biological importance and structural roles of these minor collagens in the cartilage matrix have not as yet been elucidated.

We have now examined human articular cartilage from the femoral head for the presence of different collagen chains, with special regard to aging and osteoarthrosis.

MATERIAL AND METHODS

Human femoral head cartilage was sampled from 18 cases of autopsy material from newborns to adults 91 years old and surgical material from 16 patients aged 45–80 years suffering from severe arthrosis of the hip, histologic-histochemical grades 6–13 according to Mankin (1973). For comparison, human and bovine costal cartilage of different age groups was also studied (eight cases). All samples were stored at 0–4° C not longer than 16 h before investigation. Collagen was solubilized from cartilage by limited pepsin digestion and fractionated by differential salt precipitation, according to Reese & Mayne (1981). The cartilage was homogenized by Polytron homogenizer and proteoglycans were extracted from the cartillage fragments by 4.0 M guanidine chloride, 3 times for 24 h. The rest of the material was washed in ice cold water and solubilized by pepsin digestion (0.1 mg Pepsin-SERVA/mg fresh cartilage) in 0.5 M acetic acid solution containing 0.2 M NaCl for 16 h at 0–4° C. The articular cartilage fragments were not solubilized completely; the solubilized collagen went up to 20–30 per cent of the fresh weight of cartilage. The neutralized supernatants after inactivation of pepsin were fractionated by differential salt precipitation: fraction 1 was obtained by 0.9 M NaCl in 0.5 M acetic acid, fraction 2 by 1.2 M NaCl in 0.5 M acetic acid, fraction 3 by 2.0 M NaCl in 0.5 M acetic acid. After centrifugation each precipitate was resuspended in 0.5 M acetic acid, dialysed against diluted acetic acid and freeze-dried *in vacuo*. Fraction 1 contained mainly collagen type II and traces of type I, fraction 2 mainly 1α, 2α and 3α collagens and fraction 3, M-collagens in addition to α chain collagens. The collagen content of the samples was measured at 230 nm, using type II collagen as standard. For separation of different collagen chains, SDS-6 per cent polyacrylamide slab gel electrophoresis was used. HMW M-collagen, 1α, 2α and 3α appeared between α_1 (II) and that of dimeric components in the order of the running whereas LMW-M collagen was close to the front line. After reduction with 2-mercaptoetanol, HMW component produced three subcomponents; those of the reduced subcomponent of LMW were not studied. For further identification of different collagen chains no other method was used. Every cartilage sample was examined histologically.

RESULTS

The articular and costal cartilage of newborn and children was white, transparent and elastic. Microscopically the cartilage was rich in cells with strong metachromatic matrix (Figure 1 E, F). In adults the costal carilage was calcified and the articular cartilage was yellowish grey. Microscopically the matrix was unevenly metachromatic with fewer cells (Figure 1 A). In osteoarthrosis three main pathologic types of cartilage were found microscopically; usually in the same femoral head (Figure 2). The middle part of the femoral heads was covered with thickened, yellowish cartilage (degenerated cartilage); in the

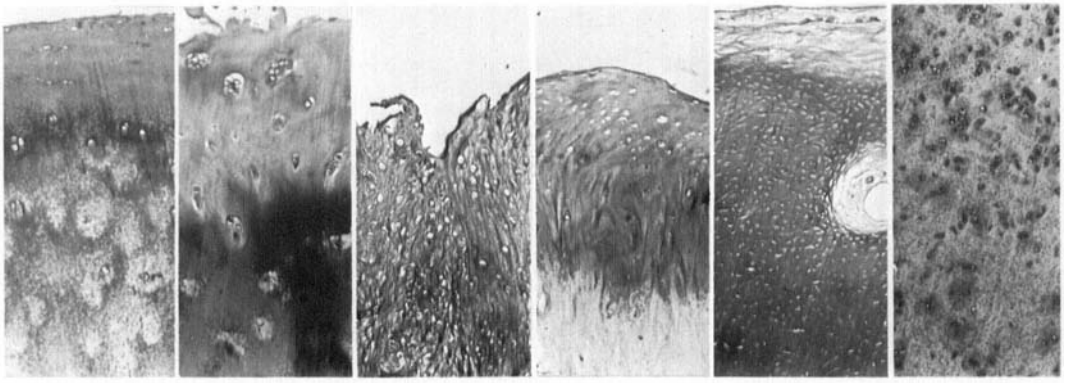

Figure 1. Microscopic picture of human articular and costosternal cartilage, Alcian-blue-PAS staining, ×25. A: *mature hyaline articular cartilage from normal femoral head.* B: *degenerated articular cartilage from arthrotic femoral head.* C: *reparative fibrocartilage from eburnated articular surface of arthrotic femoral head.* D: *fibrocartilage from surface of osteophyte of arthrotic femoral head.* E: *immature articular cartilage from femoral head of newborn.* F: *immature costal cartilage from newborn.*

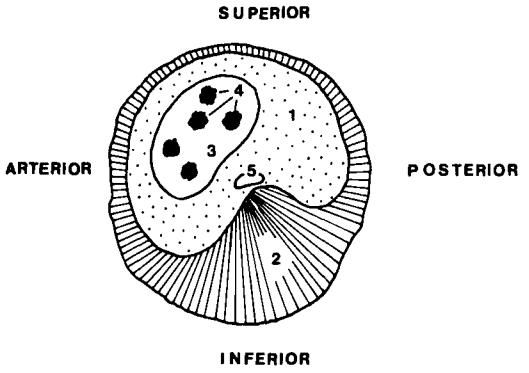

Figure 2. The surface of an osteoarthrotic femoral head, 1 degenerated articular cartilage, 2 osteophyte, 3 eburnated surface, 4 islets of reparative cartilage, 5 fovea capitis femoris.

peripheral part of the femoral heads rigid, white cartilage was found covering the osteophytes (fibrocartilage), whereas the central part of the femoral heads was worn down, eburnated with white, soft, cartilage islets in it (reparative cartilage). Microscopically the degenerated cartilage had irregular, less metachromatic matrix with a substance poor in cells; superficially the cells were distributed in clumps or clones. The reparative cartilage looked like fibrous connective tissue with a few rounded and slightly elongated chondrocytes as cell elements. In the superficial layer of the fibrocartilage forming the surface of the osteophytes, elongated cells parallel to the surface could be found. In the deeper layer a fibrous structure dominated in which the cells appeared partly dispersed or separate, partly arranged in groups. The metachromasia of the matrix was of changing intensity and arrangement.

The distribution in percentage of collagen in fractions of pepsin-solubilized collagen varied with age and with that of the pathological type of cartilage (Table 1). The minor collagens (fractions 2 and 3) decreased with age from 14 per cent to 3 per cent. The HMW component of M-collagen disappeared completely with age; in some elderly individuals an increase of LMW M-components was found in macroscopically normal cartilage. In arthrotic cartilage minor collagens increased from 2 per cent to 8 per cent depending on the type of pathologic changes. In reparative cartilage fraction 2 (α components) and in fibrocartilage fraction 3 (α components + M-collagen) were more abundant than in normal cartilage.

The above changes could be demonstrated by the electrophoretic pattern of different collagen fractions. In costal cartilage of newborn HMW M-components were elevated together with 1α, 2α and 3α bands. After reduction with 2-mercaptoethanol the HMW M-component gave rise to three subcomponents. The early adult costal cartilage contained only LMW M-components (Figure 3). In femoral cartilage the α components

Table 1. The distribution of pepsin-solubilized human cartilage collagen fractions

Sample Cartilage	Fraction 1 Type II Type I (in traces) per cent	Fraction 2 1-α 2-α 3-α per cent	Fraction 3 1-α 2-α 3-α + M-coll. per cent
Autopsy material			
Newborn sterno-costal	82	14.5	3.5
Young adult sterno-costal	89	6.4	3.8
Child femoral head	97	2.8	0.2
Adult femoral head	98	2.2	0.2
Surgically removed osteoarthrotic femoral heads			
Degenerated	97	2.7	0.3
Fibrocartilage	95	4.0	1.0
Reparative cartilage	92	8.8	1.2

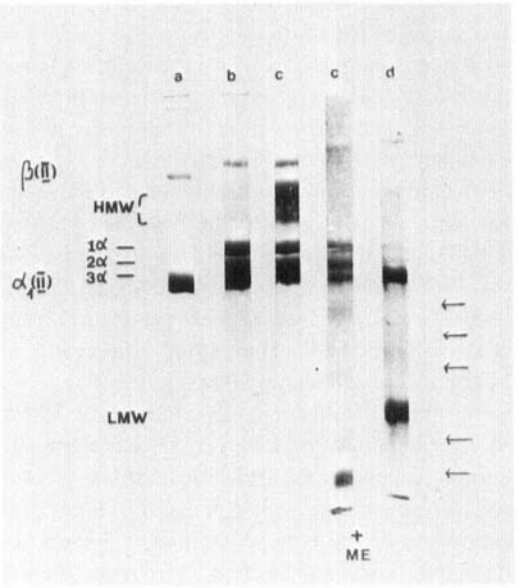

Figure 3. SDS-6 per cent polyacrylamide slab gel electrophoresis of fraction 3 of cartilage collagen obtained after salt precipitation (2.0 M NaCl–0.5 M HOAc) from human costosternal and articular cartilage (autopsy material) without and with reduction with 2-mercaptoetanol (+ME). a: *collagen type II as standard,* b: *femoral head and* c: *costosternal cartilage from a 2 months old infant,* d: *costosternal cartilage of a 31-year-old woman.* HMW: *high molecular weight and* LMW: *low molecular weight components of M-collagen. Arrows on the right side indicate the bands of subcomponents of HMW.*

decreased with age; no HMW M-components could be found, and the LMW M-component present in cartilage of children, was generally no longer detectable in adults (Figure 4). In arthrotic cartilage of the femoral head, the increase of α chain components and the reappearance of HMW and LMW M-components were seen, the

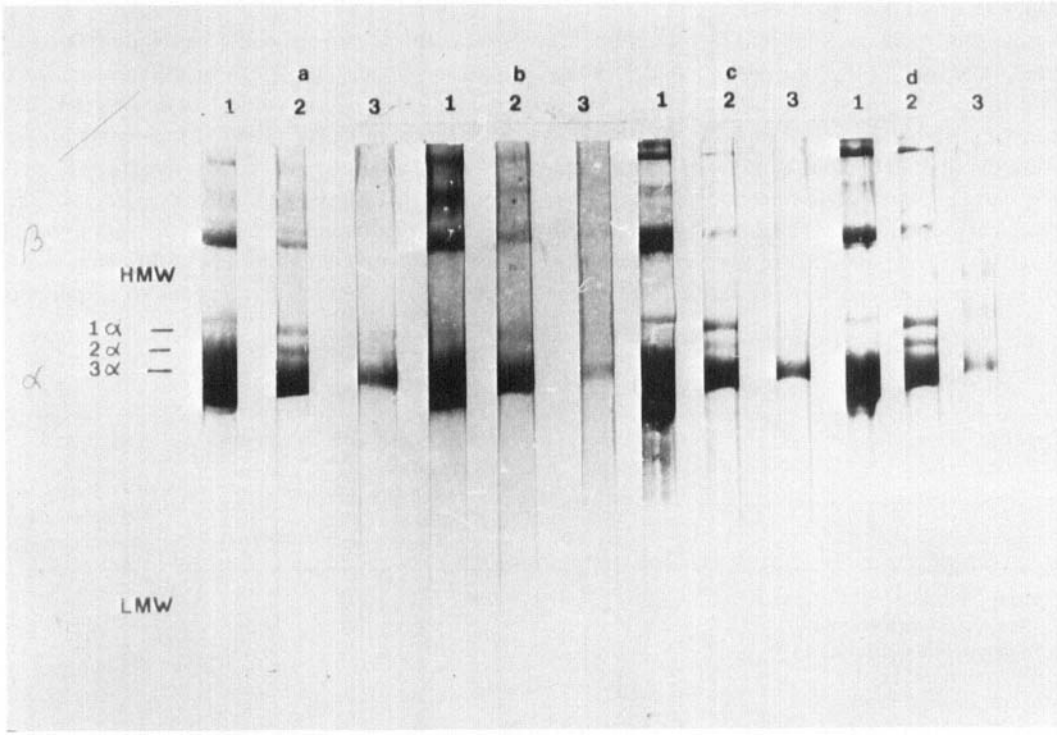

Figure 4. SDS-6 per cent polyacrylamide slab gel electrophosesis cartilage of collagen fractions of human femoral head obtained by salt precipitation. 1: *precipitate at 0.9 NaCl–0.5 M HOAc,* 2: *at 1.2 M NaCl/0.5 HOAc and* 3: *at 2.0 M NaCl/0.5 M HOAc.* a: *1-month-old infant,* b: *72-year-old man with "normal" cartilage,* c *and* d: *the degenerated and fibrocartilage, respectively from a 65-year-old woman with osteoarthrosis.* HMW: *high molecular weight and* LMW: *low molecular weight components of M-collagen.*

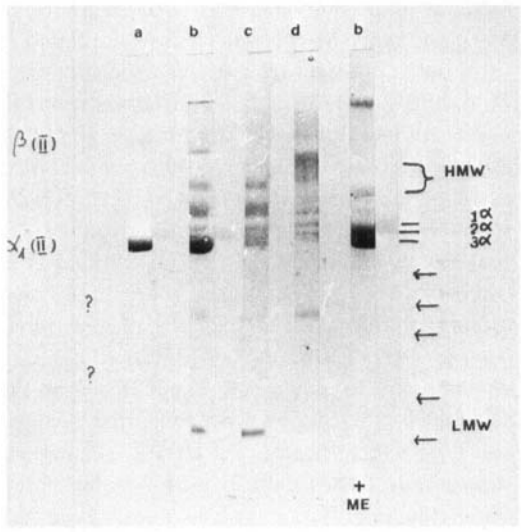

Figure 5. SDS-6 per cent polyacrylamide slab gel electrophoresis of fibrotic osteoarthrotic cartilage collagen fraction 3 (precipitate at 2.0 NaCl/0.5 M HOAc) without and with reduction with 2-mercaptoethanol treatment (+ME). a: *collagen type II as standard,* b: *53,* c: *72 and* d: *60-year-old patients.* HMW: *high molecular weight and* LMW: *low molecular weight components of M-collagen. Arrows on the right side indicate the bands of subcomponents of HMW collagen after reduction, question marks on the left show the positions of unidentified bands.*

latter two in fibrocartilage (Figures 4 and 5). In reparative cartilage the α components were elevated. The HMW components consisted of two bands; in some cases the slower running band was broader; in most of the cases the faster band was greater. In all cases of fibrocartilage between 3α and LMW M-components subcomponents were found (unidentified bands marked by question-marks in Figure 5). After reduction the bands disappeared and new subcomponents appeared marked by arrows in the figures. At the moment we could find no correlation between the type of HMW M-components and the histological changes. The two bands of HMW and subcomponents could be pepsin degrated products as well. Based on our results we propose that the changes in human articular cartilage with age and osteoarthrosis may be characterized according to the minor collagen profiles (Table 2).

DISCUSSION

The heterogeneity of human costal and articular collagen of cartilage was described by Burgeson & Hollister (1979). In their fundamental paper only a short remark was devoted to the age

Table 2. SDS-Page pattern of minor collagens (fraction 2- and 3)

	Normal articular cartilage		Arthrotic cartilage		
	Children	Adult	Degenerated	Fibrotic	Reparative
Start					
γ(II)					
β(II)					
HMW				▨	
1-α	▬	- - - -	▬	▬	▬
2-α	▬	- - - -	▬	▬	▬
3-α	▬	———	▬	▬	▬
α(II)				- - - -	
				- - - -	
LMW	———	- - - -	- - - -	———	
front line					

changes of these cartilage collagens, while no mention was made of pathologic changes. Most of the data dealing with the change of collagen in osteoarthrotic cartilage concern the change in main types of collagen based on the supposed understanding of normal hyaline cartilage composition. The main pathological change in composition was the shift of collagen production from type II to I (Goldwasser et al. 1982), the higher solubility of arthrotic cartilage (Herbage 1975, Adam et al. 1976), in experimental osteoarthrosis the increase of triple helical, γ components observed after reduction as α chain components (Lust & Miller 1980). The grading of articular cartilage with age and in arthrosis based on the histological finding was proposed by Mankin (1973). In the present paper we studied the aging and the arthrotic changes of articular cartilage with special regard to the recently described minor collagens of cartilage. These components were overlooked earlier because of their high solubility. The minor collagen chains differ from type II collagen and are similar but distinguishable from collagen type V. Gay et al. (1981) emphasized that human hyaline cartilage contains exclusively α_1 (V) chains with a pericellular localization. However, Reese & Mayne (1981) have established by CNBr and peptide mapping that the chicken cartilage 1α and 2α are similar to but different from α_1 (V) and α_2 (V) chains as found in human cartilage by Burgeson et al. (1982). We found that in human articular and costal cartilage the minor collagen component decreased and the HMW component disappeared with age. In adult articular cartilage, the minor collagens are 1α, 2α and 3α chains in reduced percentage 3–4 per cent, and the main collagen component is the type II collagen. In osteoarthrosis, in the degenerated, fibrotic and reparative cartilage, an increase of 1α, 2α and 3α components was observed. In fibrotic cartilage M-components were found. On the basis of all this we propose to characterize the arthrotic cartilage on biochemical findings according to the pattern of minor collagens of the solubilized collagen extract.

The main change in collagen metabolism during osteoarthrosis seems to be the turning back to the immature stage of hyaline cartilage with the increase of α components and reappearance of M-components. M-collagen chains were found to form fibrils different from native collagen (Ayad et al. 1981) and 1α, 2α and 3α chains failed to aggregate into fibrils (von der Mark et al. 1982). Although Reese & Mayne (1981) stated that cartilage matrix requires minor collagen as well, in addition to type collagen II, one can assume that the change in collagen chain-distribution in osteoarthrosis worsens the structural and biomechanical characteristics of collagen fibres. The proper distribution of different collagen chains seems to play a determinative role in the structure of matrix. It was found that Arteparon®, an oversulphated chondroitin sulphate, a drug administered locally in arthrotic joints, influences the distribution and synthesis (von der Mark 1980) of minor collagens. In chondrocyte culture the secretion of minor collagens into the medium increased as an effect of Arteparon (Németh-Csóka & von der Mark 1982). In the present stage of our investigation we emphasize the importance of the analysis of different collagenous chains in cartilage because the distribution of different collagens was found to be a good indicator for judgment of physiologic and pathologic changes.

ACKNOWLEDGEMENTS

We thank Antonia Szabo for her technical assistance in chemical investigation. This work was supported by the Scientific Council of the Hungarian National Health Service under Code Number 03/7-09/541.

REFERENCES

Adam, M., Musilova, J. & Deyl, Z. (1976) Cartilage collagen in osteoarthrosis. *Clin. Chim. Acta* **69**, 53–59.

Ayad, S., Abedin, M. Z., Grundy, S. M. & Weiss, J. B. (1981) Isolation and characterisation of an unusual collagen from hyalin cartilage and intervertebral disc. *FEBS Lett.* **123**, 195–199.

Burgeson, R. E. & Hollister, D. W. (1979) Collagen heterogeneity in human cartilage: identification of several new collagen chains. *Biochem. Biophys. Res. Commun.* **87**, 1124–1131.

Burgeson, R. E., Hebda, P. A., Morris, N. P. & Hollister, D. W. (1982) Human cartilage collagens. Comparison of cartilage collagens with human type V collagen. *J. Biol. Chem.* **257**, 7852–7856.

Gay, S., Rhodes, R. K., Gay, R. E. & Miller, E. J. (1981) Collagen molecules comprised of α_1 (V) – chains (β-chains): An apparent localization in the exocytoskeleton. *Coll. Res.* **1**, 53–58.

Goldwasser, M., Astley, T., van der Rest, M. & Glorieux, F. H. (1982) Analysis of the type of collagen present in osteoarthritic human cartilage. *Clin. Orthop.* **167**, 296–302.

Herbage, D. (1975) Chain composition of pepsin-solubilized collagen from human normal and osteoarthritic cartilage. *Ann. Rheum. Dis.* (Suppl.) **34**, 35–40.

Lust, G. & Miller, D. (1980) Biochemical changes in canine osteoarthrosis. In *The aetiopathogenesis of osteoarthrosis,* Pitman Medical, London. pp. 47–51.

Manking, H. J. (1973) Biochemical and metabolic abnormalities in osteoarthritic human cartilage. *Fed. Proc.* **32**, 1478–1483.

Németh-Csóka, M. & von der Mark, K. (1983) The effect of Arteparon upon heterogenous collagen chains secretion in chondrocyte tissue culture. In press.

Reese, C. A. & Mayne, R. (1981) Minor collagens of chicken hyaline cartilage. *Biochemistry* **20**, 5443–5449.

Reese, C. A., Wiedemann, H., Kühn, K. & Mayne, R. (1982) Characterisation of a highly soluble collagenous molecule isolated from chicken hyaline cartilage. *Biochemistry.* **21**, 826–830.

Shimokomaki, M., Duance, V. C. & Bailey, A. (1980) Identification of a new disulphide bonded collagen from cartilage. *FEBS Lett.* **121**, 51–54.

von der Mark, K. (1979) Kollagensynthese in Chondrocyten-Kulturen unter dem Einfluss von Arteparon. *IX. Europäischer Kongress für Rheumatologia,* Wiesbaden, Congress Series: Internationales Arzneimittel-symposium Arteparon, pp. 39–50. *Euler, Basel.*

von der Mark, K., von Menzel, M. & Wiedemann, H. (1982) Isolation and characterisation of new collagens from chick cartilage. *Eur. J. Biochem.* **124**, 54–62.

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