

## Collagen, elastin and glycosaminoglycans in aortic aneurysms

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The walls of human abdominal aortas and atherosclerosis-induced aneurysms contain similar amounts of collagen. The quantitative ratio between collagens of various types of this protein does not differ significantly either, whereas solubility of the collagen in aneurysmal wall and its susceptibility to the action of EDTA are distinctly decreased. In contrast with collagen, the amount of elastin in aneurysms is significantly lower. Total amount of glycosaminoglycans slightly decreased as compared with that of normal tissue, but the ratio of particular compounds varies. The percentage of chondroitin sulphate is increased and that of heparan sulphate significantly decreased. The significance of these changes in pathogenesis of aneurysms is discussed.

Extracellular matrix (ECM) components contribute essentially for normal functioning of the arterial wall. Not only mechanical demands of this tissue are fulfilled by ECM structures, but also a broad spectrum of other functions is completely or partly covered by the extracellular matrix. ECM play a role in intra- and transvascular diffusion of water, ions and various metabolites. Some of them participate in induction of platelet aggregation, regulation of cellular activities, induction of chemotaxis, anchoring of cells within the matrix, binding of ions and cytokines, etc. (for review see [1, 2]). Alterations in the aortic ECM have been proposed to explain the formation of atherosclerotic aneurysms [3].

Collagen and elastin are major structural proteins in vascular connective tissue. They make a major contribution to the tensile strength and

elasticity of the vessel. It has been shown that treatment *in vitro* of an artery with elastase or collagenase reduces its pressure resistance [4]. Some heritable collagen disorders are accompanied by ruptures of the arteries, haemorrhagic disorders and arterial aneurysms [5-7]. In fibroblasts of some patients with cerebral artery aneurysm cultured *in vitro* the rate of collagen synthesis was reduced [8]. Deficiencies of total collagen, type III collagen and elastin have been proposed to explain aneurysm formation. The atherosclerotic process is accompanied by significant changes in glycosaminoglycans (GAGs) of the arterial wall [9, 10]. No doubt, these alterations result in distinct changes of physiological processes occurring in the arterial wall [1, 2], including modulation of collagen fibrillogenesis [11]. In many cases abdominal aortic aneurysms are

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Abbreviations used: Ch4S, chondroitin-4-sulphate; Ch6S, chondroitin-6-sulphate; DS, dermatan sulphate; ECM, extracellular matrix; GAG, glycosaminoglycan; HA, hyaluronic acid; Hep, heparin; HS, heparan sulphate; KS, keratan sulphate.

associated with atherosclerosis [3]. We have decided to study the collagen, elastin and GAG contents, solubility of collagen and heterogeneity of collagen and GAGs in aneurysmal walls in comparison with those of control aortas taken from kidney donors.

## MATERIALS AND METHODS

**Tissue material.** Studies were performed on atherosclerotic aneurysms of the abdominal aorta dissected from 13 patients (12 men and 1 woman, 52–74 years of age) during surgical operations, and samples of abdominal aorta taken from 7 kidney donors without symptoms of atherosclerosis (men, 23–44 years of age). The sections of aneurysms and aortas were separated from adjacent tissues and submitted to further examination.

**Collagen contents** in individual samples of aneurysm and aorta walls were determined by the assay of hydroxyproline as described by Woessner [12]. Since hydroxyproline constitutes about 1/8 (w/w) of collagen weight, the approximate amount of this protein in the investigated tissues was calculated by multiplication of hydroxyproline content by 8. Mean values  $\pm$  standard deviations (S.D.) were calculated.

**The collagen solubility assay** was performed as described in a previous paper [13].

**Fractionation of collagen** was performed by the method of Murata [14] as described in detail in our previous paper [13]. The isolated fractions were submitted to electrophoresis on sodium dodecyl sulphate/8% polyacrylamide gel as described by Laemmli [15]. Since type III collagen contains disulphide bonds between alpha-subunits this sample was submitted to reduction with dithiothreitol. The electrophoretic mobilities of both nonreduced and reduced material were compared. The quantities of particular collagen types were expressed as percentage of total collagen content.

**Elastin** was isolated and quantitatively determined by the method described by Robert [16].

**Contents of glycosaminoglycans** (except keratan sulphate) were measured by the assay of uronic acids [17]. Keratan sulphate was estimated by the assay of hexosamines [18].

**Isolation and fractionation of glycosaminoglycans** were performed by the methods of Wo-

sicki [19] and Svejcar and van Robertson [20], respectively, as are described in a previous paper [21].

**Statistical analysis.** Mean values  $\pm$  standard deviations (S.D.) were calculated from individual assays in control aortas and investigated aneurysms. The results were submitted to statistical analysis with the use of Student's *t* test, accepting  $P < 0.05$  as significant.

## RESULTS

It can be seen from Fig. 1 that the walls of normal aortas and aneurysms contain similar amounts of collagen: 0.37 mg and 0.41 mg per mg of dry tissue, respectively. The differences were statistically insignificant.

The solubility of collagen from the aneurysms was lower in comparison with that of control aortas. By the use of 1 M NaCl and of 0.15 M citrate (pH 3.7) about 10% of total collagen was extracted from normal aortas and only 2% from the aneurysms. A part of polymeric (insoluble) collagen of control aortas was depolymerized in 4% EDTA and became soluble in 0.2 M acetic acid. In contrast, only trace amounts of insoluble collagen present in the investigated aneurysms were susceptible to the action of EDTA. The amount of insoluble (polymeric) collagen in the aneurysms was distinctly higher than in control aortas (Fig. 2).

When the homogenates of control aortas and aneurysms were digested with pepsin in acetic acid about 70% of total collagen was solubilized. The pepsin extracts were subsequently

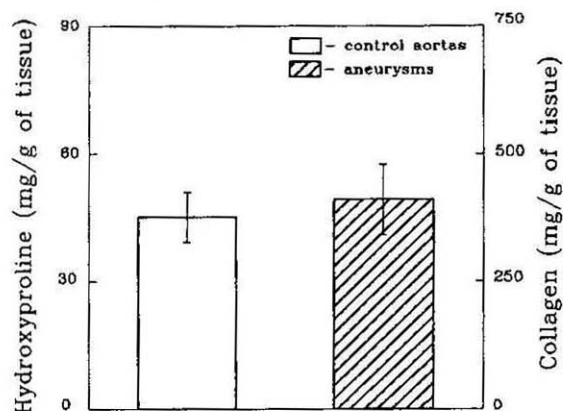


Fig. 1. Collagen content in the walls of control aortas and in those of aneurysms.

Collagen content was calculated from hydroxyproline content and expressed in mg/g of dry tissue.

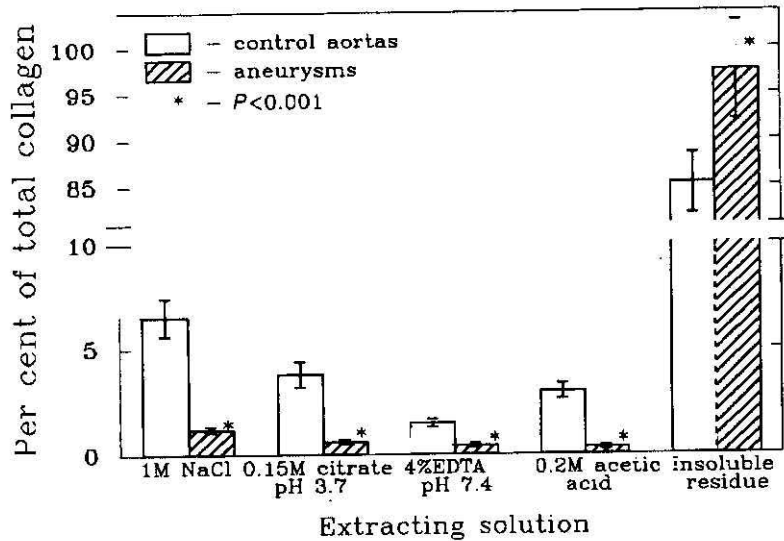


Fig. 2. Solubility of collagen from control abdominal aortas and from the aneurysms.

submitted to specific fractionation to yield three main collagen fractions with electrophoretic mobilities typical of types I, III and V collagens (Fig. 3).

The content of collagens of various type is presented in Fig. 4. Type I collagen was found to be the most abundant of both the aortic and aneurysmal collagens, either in both cases constituting about 60% of total collagen. Type III and type V collagens constituted about 22% and 17% of total collagen, respectively. No significant differences between aortas and aneurysms were found (Fig. 4).

In contrast to collagen the amount of elastin in aneurysms was three times lower in comparison to aortas (Fig. 5).

It can be seen from Fig. 6 that aneurysms contain slightly lower amounts of glycosami-

noglycans than aortas. One gram of dry, defatted aortal and aneurysmal tissue contains 9.5 mg and 7.9 mg of GAG-bound uronic acids, respectively. The quantitative differences between aortas and aneurysms are statistically significant ( $P < 0.05$ ).

The isolated GAGs were submitted to fractionation on a CF11 cellulose microcolumn and were found to show significant heterogeneity. Both control aortas and aneurysms contained several GAGs (Fig. 7). Chondroitin-4-sulphate (Ch4S) was the main component, in control aortas its mean value exceeded 31% and in aneurysms 38% of the total GAG content.

Furthermore, both tissues contained keratan sulphate (KS), hyaluronic acid (HA), heparan sulphate (HS), chondroitin-6-sulphate (Ch6S), dermatan sulphate (DS) and heparin (Hep).

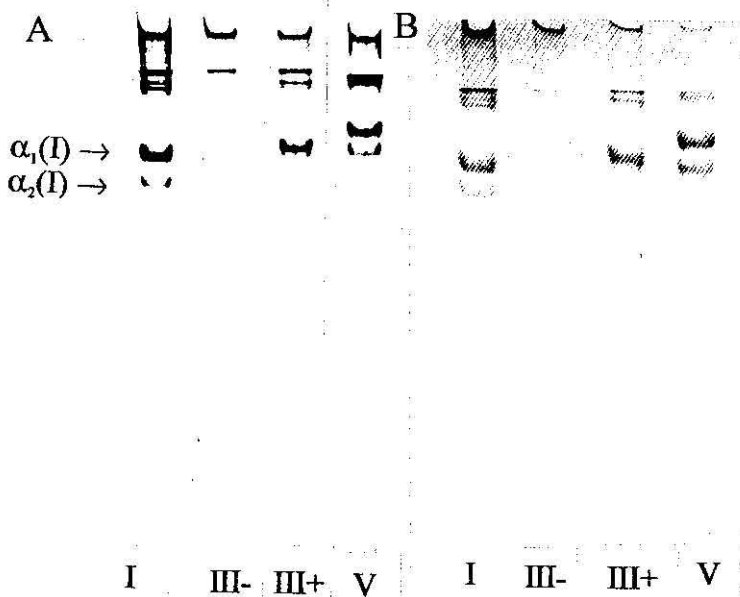


Fig. 3. Sodium dodecyl sulphate/polyacrylamide gel electrophoresis of various collagen types isolated from control aortas (A). The same electrophoretic pattern was observed in the case of collagen isolated from aneurysms (B).

Types of collagen are marked at the bottom. The position of  $\alpha_1(I)$  and  $\alpha_2(I)$  subunits are marked in the left margin. Non-reduced type III collagen does not penetrate the gel. Only trace amount of  $\alpha_1(III)$  is visible. Reduction of disulphide bonds results in appearance of this subunit. Type V collagen subunits:  $\alpha_1(V)$  and  $\alpha_2(V)$  show lower electrophoretic mobilities due to their higher relative molecular mass. (-) Without reduction, (+) reduced with dithiothreitol.

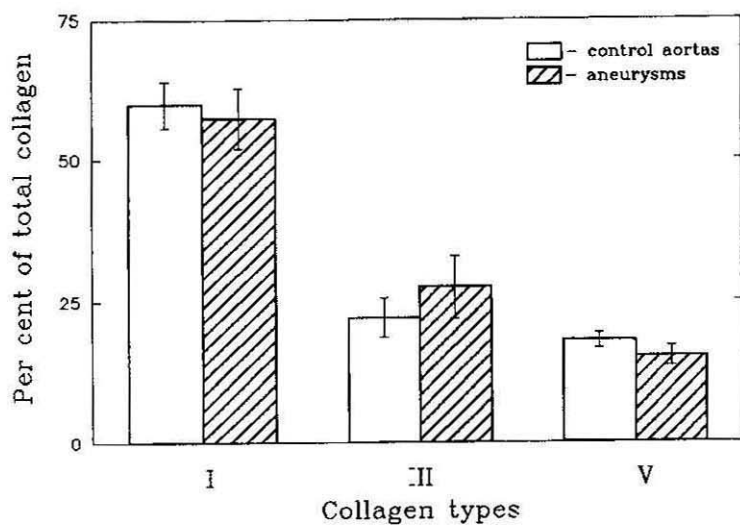


Fig. 4. Quantitative ratio of different collagen types in aortas and aneurysms.

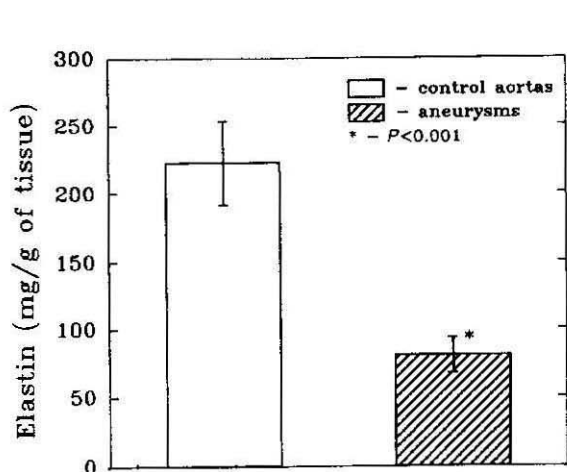


Fig. 5. Elastin content in the walls of control aortas and in those of aneurysms (mg/g of dry tissue).

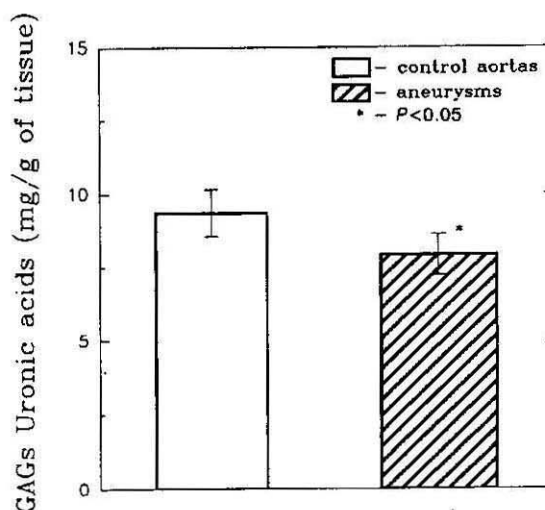


Fig. 6. Total amounts of glycosaminoglycans in aortas and aneurysms.

Some differences between the particular GAGs contents of control aortas and investigated aneurysms were observed: the aneurysms contained a significantly lower amount of heparan

sulphate and higher amount of chondroitin-4-sulphate. The differences in the amounts of other GAGs were insignificant (Fig. 7).

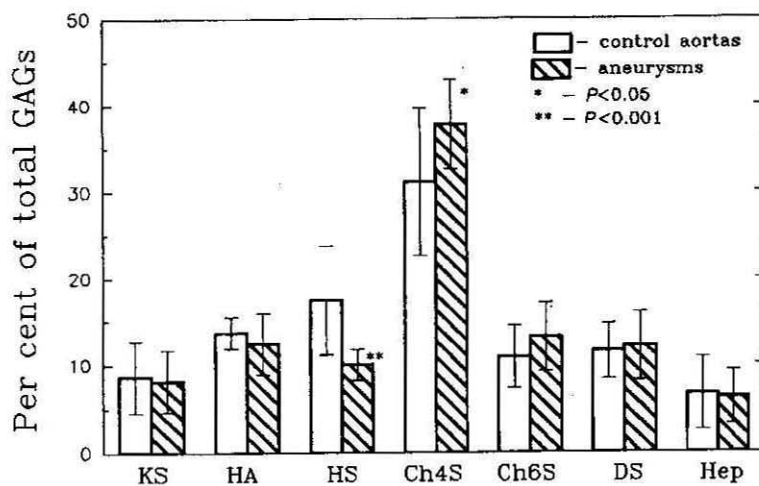


Fig. 7. Relative amounts of various glycosaminoglycans in aortas and aneurysms.

KS, keratan sulphate; HA, hyaluronic acid; HS, heparan sulphate; Ch4S, chondroitin-4-sulphate; Ch6S, chondroitin-6-sulphate; DS, dermatan sulphate; Hep, heparin.

It is of interest that the GAGs : collagen ratio in the aneurysms was significantly lower than in the control aortas (Fig. 8).

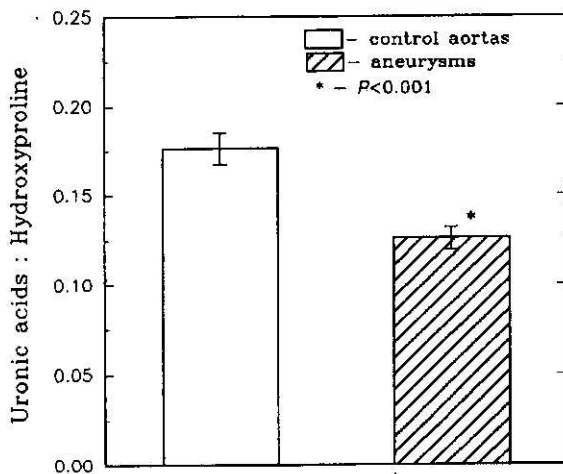


Fig. 8. Glycosaminoglycan : collagen ratio in aortas and aneurysms (expressed as mg of uronic acids per mg of hydroxyproline).

## DISCUSSION

It is commonly known that the composition of extracellular matrix changes during the ageing process [22, 23] and the composition of arterial walls is affected by atherosclerosis [9, 10, 14]. We realize that the age of our control subjects does not correspond to the age of the investigated patients with aneurysms. The control group consisted of young subjects, 23–44 years of age, whereas the patients of the investigated group were distinctly older (52–74 years). Atherosclerosis is a very common pathological syndrome which accompanies the ageing process. That is why it was impossible to collect a group of human subjects above 50 years of age free from atherosclerotic processes in the aortas. These changes in composition of the aneurysmal walls in comparison to control aortas, observed in our studies, should be attributed both to the ageing and atherosclerotic processes.

Collagen and elastin are the major ECM proteins responsible for structural integrity of aorta. Interstitial collagen types I and III represent the main portion of aortic collagen. In healthy aortas these ECM-proteins maintain the elastic aortic wall in a state resistant to aneurysmal degeneration. Alterations of the aortic ECM, especially an imbalance between

synthesis and degradation of arterial connective tissue, have been proposed to explain the formation of atherosclerotic aneurysms [3]. In atherosclerotic abdominal aneurysms collagen has been found to be deficient [24], unchanged [25], or increased [26].

According to our observations the walls of aortas and aneurysms contain similar amounts of collagen and the relationships between collagens of various type are also similar. On the other hand, the solubility of collagen contained in aneurysmal wall and its susceptibility to the action of EDTA are distinctly lower. In contrast to collagen, the amount of elastin in aneurysms is significantly lower in comparison to control aortas. The total amount of GAGs is slightly lower but the relative content of them are distinctly changed. The percentage of chondroitin sulphates are distinctly higher and that of heparan sulphate significantly lower.

It is well known that ageing is accompanied by a decrease in collagen solubility. Such a decrease has been attributed to an increase in intermolecular cross-linking between collagen molecules [27, 28]. Aldehyde groups, known to be essential for formation of cross-links, are formed by the action of lysyl oxidase which catalyses oxidative deamination of specific, peptide-bound residues of lysine and hydroxylysine to aldehydes: allysine and hydroxyallysine. The aldehydes undergo a series of condensation reactions to form intra- and inter-molecular cross-linking compounds [29].

Some proteoglycans affect the process of fibril formation [11, 30, 31]. According to Scott [11, 31] chondroitin sulphate proteoglycans interact strongly with type I collagen, electrostatically *via* glycosaminoglycan chains and by protein-protein interactions, and they inhibit fibrillogenesis. We have found that the aneurysmal walls contain relatively more of chondroitin sulphates than the walls of control aortas. The increase in chondroitin sulphates content may evoke defective collagen fibrillogenesis. Such a defect, accompanied by the decrease in elastin content, may cause reduced mechanical resistance of the arterial wall and predispose some human subjects to the development of aneurysms.

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