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Modifications of the Extracellular Matrix of Aneurysmal Abdominal Aortas as a Function of Their Size*

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Collagen and elastin are the main extracellular matrix proteins providing the aortic wall with adequate mechanical properties and resistance for proper function. Our study aimed at investigating the relationship between the elastin concentration of the wall of normal and aneurysmal abdominal aortas (AAA), the collagen concentration, and its extractibility, as a function of their size. Infrarenal aortas were collected from 30 patients undergoing operative repair of abdominal aortic aneurysm. Age-matched control samples were obtained from eight autopsies of individuals without vascular disease. Samples were divided into five groups according to the aortic diameter: control group (group N, n = 8); <50 mm (group I, n = 6); between 50–75 mm (group II, n = 10); >75 mm (group III, n = 7); and ruptured (group IV, n = 7). The collagen concentration in samples from group I was similar to the controls. An increased collagen concentration was observed in group II and remained at the same level in the largest and ruptured aneurysms. Extractibility of collagen was found to be increased in group III and was even higher in group IV. A highly significant reduction in elastin concentration was observed in group I and there was progressive reduction with increasing diameter and rupture. A significant correlation could be established between aortic diameter, increased collagen extractibility and decreased elastin content.

Key Words: Aortic aneurysms; Ruptured AAA; Extracellular matrix; Elastin; Collagen.

Introduction

Despite numerous studies, the mechanisms underlying the development of abdominal aortic aneurysms (AAA) remain unclear. Alterations or degradation of matrix proteins in the aortic wall have been reported to occur in AAA as compared to normal or atherosclerotic occlusive aortas. A consistent finding is a substantial loss of elastin demonstrated both biochemically and histochemically.[1–3] The collagen content has been reported to be diminished,[1] unaltered[4] or increased.[5] These alterations have been attributed to a disturbance in the balance of proteolytic[5,7] and anti-proteolytic activities.[8–9] The correlation between the size of the aneurysm, an index of its evolution[10] and the changes in the composition of the main extracellular matrix proteins of the aortic wall has never been described. In an attempt to define a sequence of events in the development of aneurysms, we investigated the relationship between the aortic diameter, the collagen concentration, its extractibility and the elastin concentration in the wall of normal aortas and AAA.

Materials and Methods

Samples collection and preparation

Full thickness aortic wall specimens were collected, 4–5 cm distal to the renal arteries, in 30 patients undergoing operative repair of AAA (23 patients for elective surgery and seven for emergency surgery for ruptured AAA) and eight individuals without vascular disease within 24 h after death. The mean age of the AAA group was 69.7 ± 8.7 years, 29 males/30 patients, while that of the control group was 70.3 ± 9.05 years, six males/eight individuals. All seven patients with ruptured AAA had free retroperitoneal

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blood at the time of laparotomy. Marfan Syndrome and Ehlers-Danlos patients as well as inflammatory aneurysms were excluded from our series of patients.

The size of the aneurysm was obtained from a preoperative ultrasound (US) and/or computed tomography (CT) scanning and the largest transverse diameter expressed in millimeters. Aortas were divided into five groups: controls (N group); <50 mm (group I); between 50–75 mm (group II); >75 mm (group III); and ruptured (group IV). A CT scan was obtained in five out of the seven ruptured aneurysms. The mean diameter for this group was 85 mm (range 65–100 mm).

Full thickness infrarenal aortas were dissected from associated fat and mural thrombus and stored at −20°C. The tissue specimens were cleaned of remaining adherent thrombus and crushed in liquid nitrogen (N_2). The powder was repeatedly washed at room temperature with distilled water to eliminate blood, lyophilised, extensively defatted by extraction with petroleum ether and dried.

Measurement of collagen concentration and extractibility

Aliquots (±100 mg) of defatted and dry powdered tissue were sequentially extracted for 24 h at 4°C with neutral buffered saline solution containing proteases inhibitors [1 M NaCl, 0.05 M Tris-HCl (pH 7.2), 0.5 mM N-ethylmaleimide, 0.5 mM phenylmethanesulfonyl fluoride and 20 mM EDTA], then with 0.5 M HAC brought to pH 2.0 with HCl and finally digested with a 50 µg/ml pepsin solution in 0.5 M HAC, pH 2.0. The extracts were collected by centrifugation at 20,000 rpm. The collagen content of each extract and of the residual material was determined from hydroxyproline measurements.[11]

Measurement of elastin content

The elastin content was determined by weighing the residual material after digestion with 0.1 M NaOH at 100°C for 45 min and several washings with distilled water. The purity of the elastin residue was checked by amino acid analysis using Gold Beckman high performance liquid chromotography (HPLC) on several control and aneurysmal samples. The most representative and abundant amino acids in elastin (glycine, alanine, proline and valine) accounted for more than 80% of the total residues as found in pure elastin.

Statistical analysis

The distribution of each variable was characterised by the mean and standard deviation. Group mean values were compared by analysis of variance and multiple comparison procedures were used to assess which patient group differed statistically. To determine the relationship between the extracellular matrix proteins and the size of the abdominal aortic aneurysm, we applied linear and quadratic regression analysis. The best model was chosen on the basis of the percentage of variance explained. All results were considered to be significant at the 5% critical level ($p < 0.05$).

Results

The mean values of the collagen and elastin concentrations in the aortic wall of the tissue specimens classified into five groups are detailed in Table 1. The results are expressed in mean percentages ± S.D. of the defatted dry weight of powdered tissue. As compared to the control group, the collagen content of the aneurysms in group I was unchanged. The increase in collagen concentration observed in the larger aneurysms (groups II and III), as well as in the group of ruptured aneurysms, was not statistically significant ($F = 1.68$, 4 and 26 d.f., $p = 0.185$). The elastin concentration, also expressed as the mean percentage (± S.D.) of defatted dry weight tissue (Table 1) was drastically and highly significantly reduced even in the group I aneurysms and further decreased in groups II, III and IV ($F = 12.7$, 4 and 33 d.f., $p < 0.0001$).

Table 1. Collagen and elastin concentration (in % of defatted dry weight) in normal and aneurysmal aortas

Group	Collagen (%)	Elastin (%)
Control	28.4 ± 6.1 ($n = 8$)	15.3 ± 6.3 ($n = 8$)
I (<50mm)	25.5 ± 7.8 ($n = 4$)	6.8 ± 3.9* ($n = 6$)
II (50–75mm)	34.8 ± 10.0 ($n = 6$)	4.4 ± 3.5* ($n = 10$)
III (>75mm)	34.8 ± 6.9 ($n = 7$)	4.6 ± 1.5* ($n = 7$)
IV (ruptured)	32.7 ± 6.6 ($n = 6$)	3.4 ± 1.6* ($n = 6$)

* Significantly different from the control group with $p < 0.05$.

The extractibility of collagen was estimated by measuring the amount of collagen solubilised by sequential extraction as described in Materials and

Methods. The mean values (± S.D.) for each group are given in Table 2. Analysis of variance applied to the data yielded a significant result ($F = 4.39$, 4 and 26 d.f., $p = 0.008$). Multiple comparisons, however, revealed that the extractibility of collagen was similar to that of the controls in all AAA groups, except for the ruptured specimens.

Table 2. Extractibility of collagen in normal and aneurysmal aortas

Group	Soluble collagen (ng/mg dry weight)
Control ($n = 8$)	461 ± 95
I (<50mm) ($n = 4$)	419 ± 116
II (50–75mm) ($n = 6$)	613 ± 233
II (>75mm) ($n = 7$)	649 ± 195
IV (ruptured) ($n = 7$)	908 ± 370*

* Significantly different from the control group with $p < 0.05$.

When individual values of elastin concentration were plotted against the respective diameter of the aorta (Fig. 1), they appeared to decrease more sharply during the early phases of the aneurysmal development; a slow down of the process was observed in larger aneurysms. This relationship was best fitted by

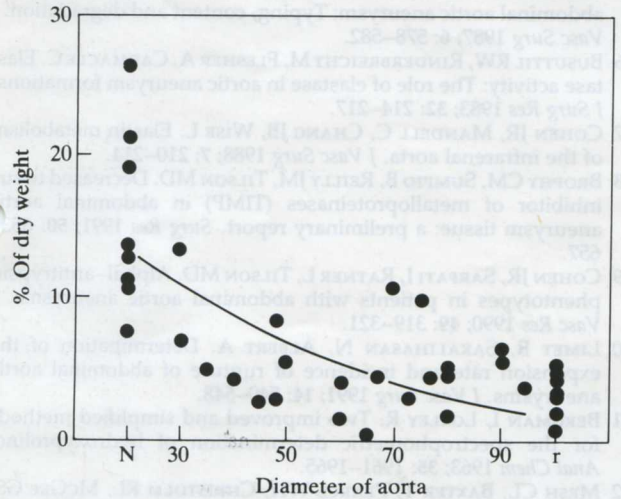

Fig. 1. Significant quadratic relationship between the elastin concentration and the individual values of the diameter of the normal aortas (N), AAA of increasing size and ruptured AAA (r).

a quadratic regression model ($y = 19.3 - 0.316x + 0.0014x$). The percentage of variance explained was 47.8% ($p < 0.0001$), whereas for the linear model it was only 28.4%. When plotted against the diameter of the aorta, the proportion of extracted collagen (Fig. 2) was found to increase linearly with the diameter of the aorta ($y = 369 + 3.48x$, $r = 0.60$, $p < 0.001$).

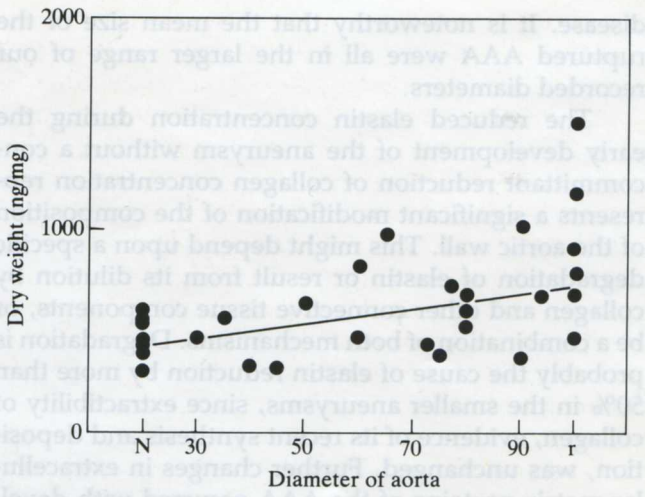

Fig. 2. Significant positive linear correlation between the collagen extractibility and the individual values of the diameter of the normal aortas (N), AAA of increasing size and ruptured AAA (r).

Discussion

During the selection of the patients, aneurysms potentially associated with known heritable connective tissue disorders such as collagen type III deficiency in Ehlers-Danlos type IV or mutations in the fibrillin genes in the Marfan syndrome were excluded from our series. Similarly, inflammatory aneurysms macroscopically recognised at time of operation were not entered in this study. However it has to be pointed out that in some samples of macroscopically non-inflammatory aneurysms, focal infiltrates of inflammatory cells were observed, as also described by Rizzo *et al.*[3]

The strategy used in this study attempted to define a sequence of events that could help to understand the mechanism(s) operating in the development of aortic aneurysms. The operational classification of the AAA into three classes, <50, 50–75 and >75 mm, was based upon our previously published observation[10] that, in longitudinal studies, the aneurysmal growth better fits an exponential model. According to this model, after a quiescent phase which can last for several years, there is a turning point after which the aneurysmal growth is accelerated. One can therefore consider that the three groups of increasing diameter are representative of the evolution of the disease with time. Rupture at any diameter can be considered as the end point of the

disease. It is noteworthy that the mean size of the ruptured AAA were all in the larger range of our recorded diameters.

The reduced elastin concentration during the early development of the aneurysm without a concommittant reduction of collagen concentration represents a significant modification of the composition of the aortic wall. This might depend upon a specific degradation of elastin or result from its dilution by collagen and other connective tissue components, or be a combination of both mechanisms. Degradation is probably the cause of elastin reduction by more than 50% in the smaller aneurysms, since extractibility of collagen, evidence of its recent synthesis and deposition, was unchanged. Further changes in extracellular matrix proteins of the AAA occurred with development of the aneurysms. Dilution by newly synthesised components possibly occurs in the later development of the disease as suggested by the increased concentration of collagen on a dry weight basis and a rise in its extractibility while elastin content was only slightly altered. This is supported by the recent observation of a higher steady-state level of collagen type I mRNA in the aortic wall of aneurysms.[12] The increased extractibility of collagen most obvious in the ruptured specimens, that might reflect an accelerated turn-over rate as discussed above, could also result from a destabilisation of the polymers after partial collagenase activity, leaving the cleavage products loosely associated to the fibers, and/or the cleavage of the intermolecular cross-links and/or the degradation of associated molecules such as decorin. A good candidate responsible for this process is the MMP–9 or 92 kilodalton collagenase that has been recently reported to be present in aneurysmal aortas.[13] Its potent elastase activity[14] could also play a role. The presence of elastolytic activity in AAA[6,2] is well established, although its exact nature is still controversial.[2, 13,17] However, the activity of an authentic instertitial collagenase I in non-ruptured specimens remains uncertain due to the inadequacy of the techniques used for its determination.[15,16] By using a reliable test on native collagen fibrils, collagenase activity was only detected in ruptured aneurysms[17] and would appear to be related to the presence of inflammatory cells.

Up to now, nothing is known about a potential involvement of the other component of the elastic fibers, the fibrillins, which have been implicated in the development of aneurysms in some forms of Marfans syndrome.[18]

Our findings of an early degradation of elastin followed by modifications of the collagen polymers support the suggestions of Dobrin *et al.*[19] that degradation of elastin plays a role in dilatation while subsequent collagen alteration could lead to rupture.

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