Deficiency of total collagen content and of deoxypyridinoline in intracranial aneurysm walls

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Abstract The tissue contents of total collagen and of 3-hydroxypyridinium cross-links, pyridinoline (PYD) and deoxypyridinoline (DPD), were measured in 15 samples of human aneurysms of Willis' Circle obtained at surgery and in 25 autopsy control samples of intracranial arteries of Willis' Circle obtained from 6 subjects who died of other causes than cerebral hemorrhage. PYD and DPD were detected fluorimetrically after HPLC separation. Total collagen content was significantly lower ($P < 0.001$) in aneurysm samples (mean ± S.E.M. 2.50 ± 0.33 nmol of α 1(I) collagen chain per mg of delipidated and dried material) than in controls (mean ± S.E.M. 3.86 ± 0.14). DPD, but not PYD, content appears to be lower in aneurysm walls. In the aneurysms, the tissue contents of PYD ranged from 212 to 587 pmol/nmol of α 1(I) collagen chain (mean ± S.E.M. 430 ± 31) while in control samples the values observed ranged from 292 to 642 (mean ± S.E.M. 471 ± 21). The tissue content of DPD was measurable only in 6 aneurysm samples (60%), ranging from 12 to 60 pmol/nmol of α 1(I) collagen chain (mean ± S.E.M. 33 ± 9), while in control samples, DPD content ranged from 30 to 123 (mean ± S.E.M. 75 ± 5).

Key words: Intracranial aneurysm; Collagen; Pyridinoline; Deoxypyridinoline

1. Introduction

The pathophysiological events related to the development of cerebral aneurysms are still poorly understood [1,2]. According to some theories, an inherited deficiency of collagen in cerebral arteries may be involved in the development of intracranial aneurysms [3–5], while other authors suggest that aneurysms could result from an acquired degeneration of cerebral arteries under the influence of arterial hypertension and atherosclerosis [6].

In fact, even if a deficiency of type III collagen was demonstrated in both arteries and skin biopsies from patients with ruptured aneurysms [2,7], and a decrease of type III collagen synthesis has been supported by other authors [4,8,9], recent studies seem to exclude a congenital defect of collagen synthesis in this pathology. Adamson et al. [6] showed that the frequencies of polymorphic variations in type III collagen gene loci were not different in affected individuals when compared to control cases, providing no evidence for a genetic predisposition to cerebral aneurysms. Moreover, in some cases of multiple familial aneurysms a type III collagen deficiency was not demonstrated [10], supporting the hypothesis that cerebral aneurysms are related to a genetic defect only in a minority of cases. Finally, Kuvaniemi et al. [11] recently performed detailed analyses of coding sequences for type III collagen in 40 patients with intracranial aneurysms and in no case were significant mutations in the regulatory sequence of the gene observed.

Recently, it was proposed that an alteration of the arterial wall matrix could play a significant role only in the formation of cerebral aneurysms, while other exogenous factors acting on the vessel wall seem to be involved in rupture of the aneurysm [12]. The major component of the arterial wall is collagen, particularly types I and III, which account for about 80–90% of total collagen content [13] and are responsible for the mechanical properties of the vessel. The stability of type I and III collagen fibrils is mainly due to the presence of intra- and intermolecular cross-links; it is generally accepted that collagen cross-linkage depends more on tissue localization than on the type of collagen, and that in tissues characterized by mechanical stress the cross-links formed via the hydroxylysine pathway, pyridinoline (PYD) and deoxypyridinoline (DPD), predominate.

Mature extracellular type I and III collagen fibrils are stabilized by covalent intermolecular cross-links formed by two main pathways, the allysine route, based on lysine aldehydes, and the hydroxyallysine route, based on hydroxylysine aldehydes. The hydroxylysine route predominates and leads to two non-reducible pyridinoline cross-links, PYD and DPD. PYD consists of three hydroxylysines, while DPD, the less abundant form, consists of two hydroxylysines and one lysine. In the hydroxylysine route, lysyl oxidase (protein lysine 6-oxidase; EC 1.4.3.13) converts the amine side chains of specific hydroxylysine residues into the corresponding aldehyde hydroxyallysine [14], the two terminal regions of the collagen molecule being the only established sites of this reaction [15–17].

Due to the above considerations, we decided to analyze both intracranial aneurysm walls and control samples from Willis' Circle in order to verify a possible difference in terms of total collagen and/or PYD and DPD contents.

2. Materials and methods

2.1. Tissue sampling

Fifteen patients with a mean age of 49 years (ranging from 18 to 64) were studied. In no case were cerebral aneurysms found in members of the patients' families, nor were collagenopathies of genetic origin. In only three cases was it possible to operate the patients before the aneurysm ruptured. The locations of the aneurysms are reported in Table 1. All the aneurysms were saccular and were cut under the surgical microscope parallel to the clip, in order to analyze the entire

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Table 1
Content of tissue collagen (TCC) and pyridinolines (PYD and DPD) in control (CS) and aneurysm samples (AS)

	TCC/dry tissue (nmol/mg)	PYD/α 1(I) coll. chain (pmol/nmol)	DPD/α 1(I) coll. chain (pmol/nmol)
CS ACA	2.54	524	96
CS ACA	4.85	351	53
CS ACA	3.87	544	88
CS ACA	4.87	436	51
CS ACA	4.19	540	87
CS ACA	4.45	530	87
CS ACoA	4.03	382	62
CS ACoA	3.26	574	93
CS ACoA	4.33	483	85
CS ICA	3.30	525	93
CS ICA	3.15	554	94
CS ICA	3.49	571	100
CS MCA	3.55	361	50
CS MCA	3.58	431	70
CS MCA	3.72	554	123
CS MCA	5.51	387	51
CS PCoA	3.67	336	47
CS PCoA	4.51	292	30
CS PCoA	3.40	399	57
CS PcoA	3.99	470	56
CS VBA	2.88	293	45
CS VBA	5.06	359	51
CS VBA	2.96	642	113
CS VBA	3.70	594	105
CS VBA	3.76	639	81
AS[a] ACoA	0.99	353	< 6
AS ACoA	1.17	319	< 6
AS ACoA	2.35	332	23
AS ACoA/MCA	0.69	212	< 6
AS[a] ICA	1.89	544	< 6
AS ICA	3.99	572	60
AS ICA/PCoA	3.51	270	19
AS MCA	2.99	480	59
AS MCA	3.64	589	12
AS MCA	1.33	477	< 6
AS PCoA	1.04	310	< 6
AS PCoA	2.27	439	25
AS PCoA	3.89	481	< 6
AS PCoA	3.41	564	< 6
AS[a] PCoA	4.41	504	< 6

[a]Unruptured aneurysm.

sample and exclude variability related to collagen structure in different sections of the aneurysm sac. Aneurysm samples were immediately washed in saline solution, in order to remove clot residuals, frozen and kept at −80°C until processed. Informed consent, giving a lengthy list of specific problems concerning the surgical procedures, was signed by the patients (or responsible persons).

Controls consisted of samples of human intracranial arteries of Willis' Circle obtained at autopsy from six subjects (mean age 60 years, range 55–66) who had died of other causes than cerebral hemorrhage. In these cases informed consent was given by the patients' relatives. Autopsies were performed within 24 h of death, the cadavers being maintained at +4°C in order to minimize tissue degradation due to proteolysis. The control samples, obtained during autopsy performed under proper magnification, were from internal carotid artery (ICA, $n=3$), middle cerebral artery (MCA, $n=4$), vertebro-basilar artery (VB, $n=5$), anterior cerebral artery (ACA, $n=6$), anterior communicating artery (ACoA, $n=3$), and posterior communicating artery (PCoA, $n=4$). All samples were carefully washed out in saline and then frozen and kept at −80°C until analysis.

2.2. Tissue analysis

After thawing, the tissue was homogenized, delipidated with chloroform:methanol 2:1 (v/v) for 18 h at 4°C, washed with abundant distilled water, dried by vacuum centrifugation, and weighed. All the delipidated and dried material (ranging from 1.17 to 6.4 mg) was then hydrolysed in 5 ml of 6 N HCl at 110°C for 18 h.

The tissue content of collagen was expressed in terms of nmol of α 1(I) collagen chain per mg of delipidated and dried material; the molarity of α 1(I) collagen chain was calculated on the basis of the hydroxyproline tissue content, expressed in nmol, assuming a ratio of hydroxyproline/α 1(I) chain approximately equal to 100 [18].

For hydroxyproline measurement [19], aliquots of the acid hydrolysate were dried by vacuum centrifugation and derivatized with dimethylaminoazobenzene sulfonyl-chloride (DABS-Cl). The derivatisate was injected (20 μl) into a C_{18} column and separation of the analyte was achieved in a gradient mode as previously described. The detection limit was 0.2 nmol/ml of acid hydrolysate and total inter-assay and intra-assay coefficients of variation (CV) were 6.3% and 9.3%, respectively. Hydroxyproline determination was performed in duplicate.

The total amounts of PYD and DPD were measured according to the method of Black et al. [20] with some modifications [21] using 500 μl of hydrolysate. The detection limit, for both analytes, was 20 pmol/ml of acid hydrolysate, corresponding to 6 pmol of analyte per nmol of α 1(I) collagen chain; total inter-assay and intra-assay CVs were 10% and 13%, respectively. All analyses were performed in duplicate.

The tissue contents of PYD and DPD were expressed in terms of pmol of analyte per nmol of α 1(I) collagen chain, in order to avoid the bias due to a possible difference of tissue content of collagen between samples.

3. Results

The chromatograms presented in Fig. 1, obtained from hydrolysate of a typical ICA control sample (A) and of two

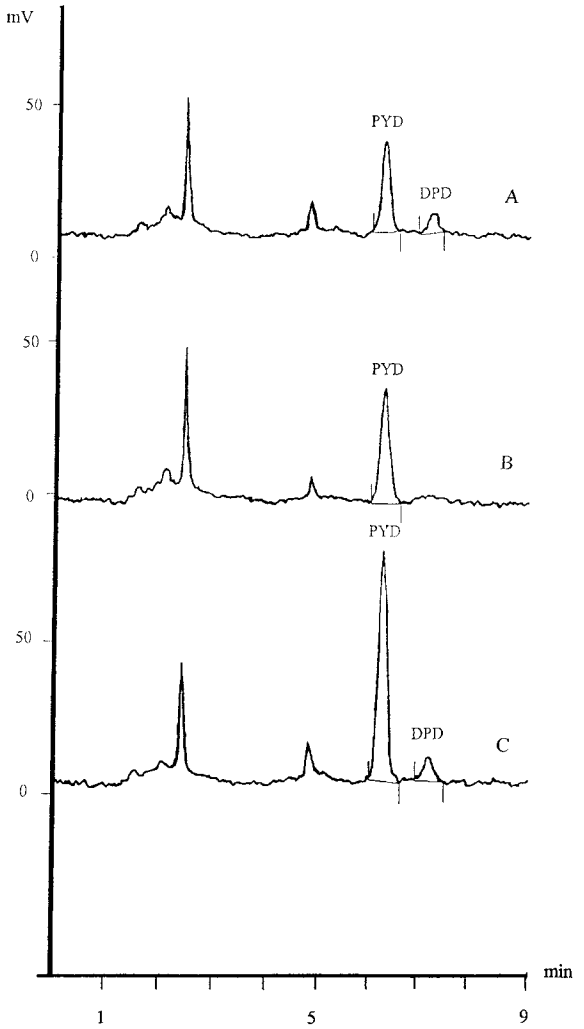

Fig. 1. Examples of HPLC separation of PYD and DPD in control (A) and aneurysm samples (B, C).

aneurysm samples (PCoA and MCA, B and C respectively), show the separation of PYD and DPD. It is noteworthy that in the chromatogram from one pathological sample (PCoA, B) the DPD peak was not detectable even when the peak area of PYD was comparable to that obtained by analyzing the hydrolysed ICA sample (A). This indicates that in some pathological samples DPD is undetectable under the used experimental conditions.

Table 1 summarizes the results obtained by analyzing the 15 aneurysm (AS) and the 25 control (CS) samples. The tissue collagen content (TCC), expressed in terms of nmol of α 1(I) collagen chain per mg of delipidated and dried material, ranged from 0.69 to 4.41 (mean ± S.E.M. 2.50 ± 0.33) in the aneurysms, and from 2.54 to 5.51 (mean ± S.E.M. 3.86 ± 0.14) in the control samples; this indicates an average lower TCC ($P < 0.001$) in the pathological samples.

The tissue content of DPD, but not of PYD, appears lower in aneurism samples than in control ones (Table 1). In particular, in the aneurysms PYD ranged from 212 to 587 pmol/nmol of α 1(I) collagen chain (mean ± S.E.M. 430 ± 31) while in control samples the values observed ranged from 292 to 642 pmol/nmol of α 1(I) collagen chain (mean ± S.E.M. 471 ± 21), with no significant difference between the two groups.

Remarkably, the tissue content of DPD was measurable in only six aneurysm samples (60%), ranging from 12 to 60 pmol/nmol of α 1(I) collagen chain (mean ± S.E.M. 33 ± 9) while in nine samples the values did not reach the minimum detectable level of 6 pmol/nmol of α 1(I) collagen chain; in the 25 control samples, DPD content ranged from 30 to 123 pmol/nmol of α 1(I) collagen chain (mean ± S.E.M. 75 ± 5). Also on the less favorable arbitrary assumption of considering equal to 6 the DPD content in the nine aneurysms with an actual value < 6 pmol/nmol of α 1(I) collagen chain, it is possible to demonstrate the presence of a highly significant difference ($P < 0.001$) in DPD content between the two groups.

4. Discussion

There are two major findings of this work: (a) the total collagen content is lower in aneurysm samples than in controls; (b) in aneurysm samples DPD (but not PYD) content is markedly lower than in control samples. The reduced amount of total collagen was not an unexpected result, since a decrease of type III collagen has already been reported to occur in cerebral aneurysm walls [2,4,7–9]. In contrast, the marked decrease of only DPD content represents a novel finding and an intriguing problem that deserves further experimental efforts to be explained. In particular, it would be interesting to investigate the lysyl-hydroxylase activity in the aneurysm tissue. The degree of lysyl-hydroxylation of procollagen chains could condition the availability of lysine residues and, therefore, the formation of DPD, which results from the condensation of two hydroxylysines and one lysine. With reference to this point, it is worth remarking that variation of lysyl-hydroxylase activity is known to occur between different tissues [22], suggesting the existence of a control mechanism based on tissue-specific variations in enzyme activity [23].

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