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Aortal collagen polymorphism in monkey and man

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Abstract. Aortal collagen typing in monkey and man showed the presence of types I, III and V in human aorta and types I and III in monkey aorta. Type III collagen was found to be a predominate type in both species. The molecular weight of type III collagen was similar in these species while type I collagen was different. Both monkey and human collagen types I and III were found to be immunogenic. Type I collagen was significantly increased while type III was decreased in human atherosclerotic plaque. Collagen typing in fatty streak remained unaltered.

Keywords. Human and monkey aorta; collagen types.

Introduction

Collagen has been reported to be the major component of human atherosclerotic plaque and may account for as much as 60% of the intimal protein (Smith, 1965). This collagen is synthesised at an abnormally high rate by plaque cells (McCullagh and Ehrhart, 1974) and accumulates within the intima as the disease progresses. Since subhuman primates are similar to man phylogenetically and metabolically (Ganguly *et al.,* 1977; Chakravarti and Kukreja, 1981), a model of advanced atherosclerosis in this species has been developed in our laboratory (Kukreja *et al.,* 1981; Chakravarti, 1982). There are no reports regarding the distribution of collagen types in the aorta of rhesus monkeys. The present study was conducted to elucidate the biochemical and immunological similarities in aortal collagen polymorphism in monkey and man.

Materials and methods

Chemicals

All the reagents used are of analytical grade. Pepsin was obtained from Sigma Chemical Company, St. Louis, Missouri, USA and diethylaminoethyl (DEAE)-cellulose-52 from Whatman Limited, England.

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Abbreviations used: DEAE, Diethylaminoethyl; SDS, sodium dodecyl sulphate.

Selection of normal aorta

Aorta samples were collected from 18 apparently normal healthy male rhesus monkeys and from 11 young human subjects who had died in traffics accidents. The body weight of rhesus monkeys was between 4-5 kg with an approximate age of 4 years and the age range for humans was between 25-35 years. At autopsy the whole aorta was opened longitudinally and examined with hand lens for fatty streaks and plaques. Those aortae which did not reveal macroscopic or microscopic lesions were employed for this study. These were washed with double distilled water and fibro-fatty adventitia was stripped off and media-intima processed for collagen separation.

Selection of atherosclerotic aorta

Atherosclerotic aorta from 5 human subjects who had died of atherosclerosis, ischaemic heart disease, hypertension and diabetes were taken. The aortae were examined with hand lens and Sudan IV dye for fatty streak and plaques and processed for collagen typing separately.

Extraction procedure

Extraction of collagen was done by the method of Moezer and Robert (1970) as modified by Robert *et al.* (1971). Briefly the aorta was cut into small fragments, delipidated with acetone and butanol, and homogenized in calcium chloride, Tris-citrate buffer, pH 7·5, using an Ultra-Turra homogeniser. This process was repeated 5-6 times. The supernatant was collected, pooled and dialysed against distilled water. After 48-72 h, the precipitate in the dialysis bag represented the crude collagen.

Purification of collagen on DEAE-cellulose-52 column chromatography

Crude collagen was purified according to the method of Timpl *et al.* (1978), where DEAE-cellulose-52 column (2·5×20 cm) was equilibrated in 2 M urea, 0·05 M Tris-HCI, pH 8·6 with a pressure of 80 mm Hg and flow rate of 10ml/h. Approximately 80 mg of protein was charged and elution was carried out with linear salt gradient from 0-0·3 M NaCl. Before undergoing chromatographic procedure, the hydroxyproline content was estimated by the method of Slagemann and Stalder (1967).

Chemical typing of collagen

Aortal collagen typing was done according to the method of McCullagh *et al.* (1980). Purity of each collagen was further confirmed by Polyacrylamide gel electrophoresis and compared with standard pattern of collagen separation already described by Sage *et al.* (1979).

Raising of antisera

Antisera against crude collagen from monkey and human aorta was raised in rabbits according to the method of Ebisu *et al.* (1978). Five hundred ug of collagen was injected subcutaneously with Freund's complete adjuvant into two rabbits. Four injections at four different sites were given fortnightly and bleeding was done after a week of the last injection. Antibodies were detected by immunodiffusion technique.

Determination of molecular weights

Molecular weights of different types of collagen were determined by sodium dodecyl sulphate (SDS) Polyacrylamide gel electrophoresis (Weber and Osborn, 1969) using ferritin, catalase, aldolase and albumin as marker proteins.

Results

The crude collagen (Tris-citrate buffer extract) was fractionated on DEAE-cellulose-52 column chromatography and four major peaks were obtained, for both human (figure 1) and monkey (figure 2) aortal collagen, out of which peak I was common in both the species. Peak III of human aortal collagen corresponded with peak II of the monkeys. A comparison of polyacrylamide gel electrophoresis of different types of collagen and different peaks of column revealed that peaks I and IV of both the species corresponded to types III and I collagen respectively. Peaks II and III of human aortal collagen corresponded to types Va and Vb collagen respectively, whereas peaks II and III of monkey aorta collagen did not correspond to any of the five known collagen types (figures 1 and 2).

Table 1 shows collagen concentration (μg/mg dry weight) in human and monkey aorta. Type III collagen was found to be in maximum concentration in the aorta of both the species. There was complete absence of type V collagen in monkey aorta while it was present in human aorta at low concentration.

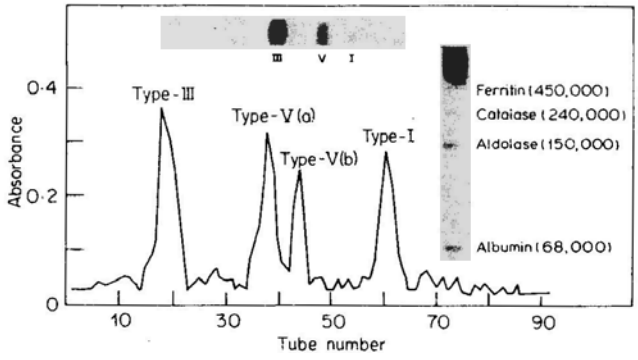

Figure 1. Human aortal collagen-DEAE-52 (2 M urea 0·05 Tris HCl buffer, pH 8·6; 0·03 M NaCl linear gradient, size 20×2·5 cm, read at 230 nm).

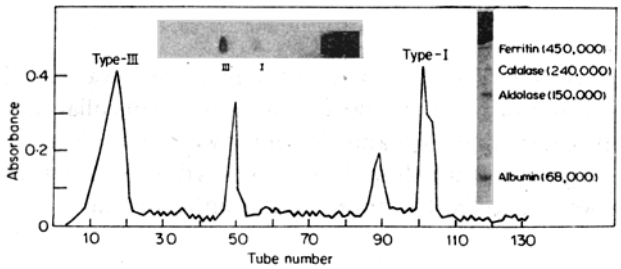

Figure 2. Monkey aortal collagen-DFAE-cellulose-52 (2 M urea 0·05 Tris HCl buffer, pH 8·6; 0·03 M NaCl linear gradient, size 20×2·5 cm, read at 230 nm).

Table 1. Collagen concentration (μg/mg dry wt).

Type	Human aorta	Monkey aorta
I	41·0± 7·2	37±5·1
III	130 ±10·7*	181±9·3*
V	29 ± 3·5	—

* $P < 0{\cdot}001$

Collagen concentration (μg/mg dry wt) in fatty streak and atherosclerotic plaque of human aorta is given in table 2. There was significant increase in type I collagen and decrease in type III collagen in fibrous plaque as compared to normal aorta. Concentration of type V collagen was slightly elevated in fibrous plaque. Collagen typing in fatty streak remained unaltered.

Molecular weight of different types of collagen is shown in table 3. Type I collagen was of a higher molecular weight than type III in the aorta of both species. Interestingly molecular weight of type III collagen from both species was found to be approximately similar.

Table 2. Collagen concentration in atherosclerotic human aorta.

	Collagen concentration (μg/mg dry wt)		
Tissue	Type I	Type III	Type V
Normal human aorta	41·0± 7·2	130 ±10·7	29 ±3·5
Fatty streak	47·3± 8·7	129·8±11·8	30·7±4·1
Fibrous plaque	91·7±10·8*	96·8± 6·5*	36·2±4·9

* $P < 0{\cdot}05$

Table 3. Molecular weights of different types of collagen through SDS Polyacrylamide gel electrophoresis.

Type	Human aorta	Monkey aorta
I	210,000	140,000
III	107,000	105,000
V	145,000	—

Antisera raised in rabbits against crude collagen from human and monkey aorta cross-reacted with different types of collagen isolated from aorta of these species. Types I and III collagen reacted with crude collagen antisera of their respective species. Type V collagen of human aorta was found to be non-immunogenic. Further, the cross reactivity of different collagen types from the aortal of monkey and man showed that type III collagen of human aorta produced precipitin line against anti-monkey aortal collagen antibodies and vice versa and showed a line of identity with each other (figures 3 and 4) while type I failed to cross-react (figure 5).

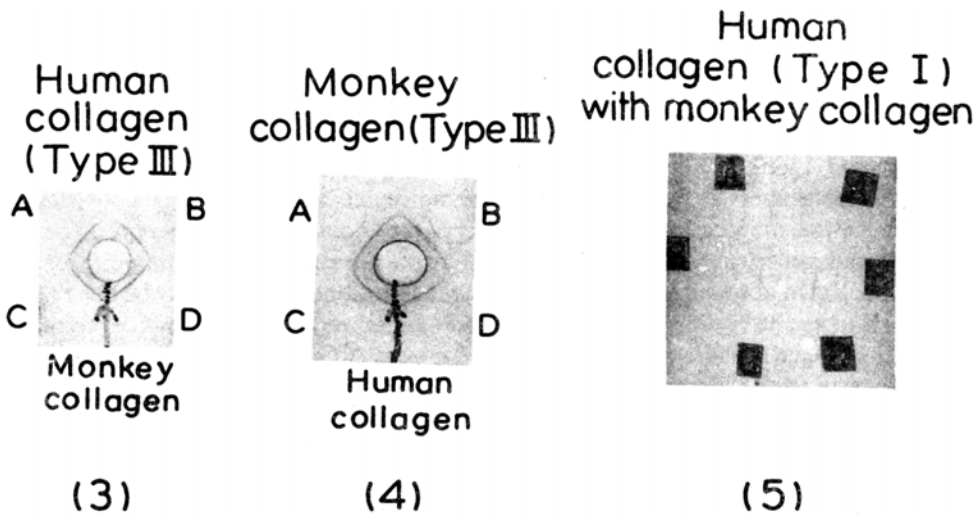

Figure 3-5. 3. Cross-reactivity between human and monkey aortal collagen type III. Antiserum against monkey aortal collagen was put into the central well and type III collagen from human aorta, skin, cartilage and tendon were put into the wells, A, B, C and D respectively. **4.** Cross-reactivity of type III collagen from human aorta with that of monkey. Antiserum against human aortal collagen was put into the central well and type III collagen from monkey aorta, skin, cartilage and tendon were put into the wells, A, B, C and D respectively. **5.** Type I collagen from human aorta failed to cross-react with antiserum of crude monkey aortal collagen. Central well contains antiserum against monkey aortal collagen and wells, A, B, C, D and E contain type I collagen from human aorta, skin, cartilage, tendon and monkey aorta respectively.

Discussion

Collagen is the major macromolecule of connective tissues and forms the structural component of both vascular and matrix tissue. Its synthesis is enhanced in atherosclerotic process by plaque cells (McCullagh and Ehrhart, 1974). Limited pepsin digestion extracted approximately 75% of the total aorta collagen and 80-90% of this extract was fractionated by salt precipitation technique (McCullagh *et al.,* 1980).

In the present study, we have observed that type III collagen concentration is maximum in human and monkey aorta. In human atherosclerotic fibrous plaque, type I collagen concentration is significantly increased while type III collagen is decreased significantly, which suggest that a major shift in the nature of aortal collagen synthesis occurs in advanced atherosclerotic plaques (McCullagh and Balian, 1975). Miller (1978) reported trace amount of type IV collagen in aortic tissue, Barnes *et al.* (1978) contradicted it. We could not obtain type IV collagen in the normal aorta or fibrous plaque of either of these species. Recent literature has revealed the presence of another type of collagen from basement membrane in the aorta which has been termed as type V (McCullagh *et al.,* 1980). We have also observed approximately 14% of type V collagen in normal human aorta and 16% in fibrous plaque respectively, but it was not detectable in the monkey aorta by the present method. However, chromatographic analysis revealed two protein peaks of monkey aortal collagen which were untypable by the salt fractionation method. There is no report in literature regarding collagen typing in monkey aorta. We have demonstrated the presence of types I and III collagen in the aorta of rhesus monkey using three different techniques *viz.* DEAE-cellulose column chromatography, polyacrylamide gel electrophoresis and limited pepsin digestion method. Absence of type V collagen in monkey aorta is an interesting finding.

To check for the similarity and dissimilarity of different types of collagen from human and monkey aorta, their molecular weights, immunogenicity and cross reactivity were assessed. It was found that the molecular weight of type III collagen in both species was similar but molecular weight of type I collagen was different. Types I and III collagens from monkey and human aorta were found to be immunogenic. Type V collagen of human aorta could not produce antibodies which may be due to its relatively low concentration in the aorta. So far as immunologic property is concerned, type III collagen of human and monkey aorta were antigenically similar, but type I collagen was quite different.

It appears that there are many similarities in the chromatographic pattern, molecular weight, antigenicity and chemical nature of human and monkey aortal collagens.

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