Histol Histopath (1986) 1: 323-326

Histology and Histopathology

The association of medial collagenous tissue with atheroma formation in the aging human aorta as revealed by a special technique

Stephen R. Greenberg
Department of Pathology, University of Health Sciences/The Chicago Medical School, North Chicago, Illinois, USA

Summary. A new technique which brilliantly colors collagen fibers in a field of polarized light reveals that during mid-life the smooth muscle cells in the tunica media of the human aorta begin to disappear. The connective tissue is divided between two regions; one below the subintimal layer and the other under the adventitia. Fine collagen fibers extend upward from the former into the subintima and beyond into the intima and the overlying atheromatous plaques of the aging aorta. Thus, the source of fibrous thickening of the vessel is not confined solely to the intimal layer; at least, a portion of the total collagen content arises deep within the aortic wall.

Key words: Aorta - Collagen - Polarizing microscopy - Aging

Introduction

The components of the central portion of the human aortic wall include smooth muscle, elastic tissue and collagen. The elastic tissue is arranged in the long axis of the vessel as a fenestrated network of fibers. The collagen content increases during the aging process at the expense of smooth muscle cells which tend to disappear in later life. (McKeown, 1965).

The relationship between the collagen in the senescent aortic media and the development of intimal atherosclerosis has not been clearly defined. It is the intent of this investigation to explore this facet of arterial disease.

Offprint requests to: Stephen R. Greenberg, Department of Pathology, University of Health Sciences, The Chicago Medical School, North Chicago, Illinois 60064, USA

Materials and methods

The aortae from 100 individuals of both sexes, aged 1 month to 78 years, were examined. All subjects, autopsied in the office of the Medical Examiner of Cook County, had succumbed to violence and were essentially free of major illnesses at the time of death.

The portion of the aorta situated between the origin of the renal arteries and the befrucation was chosen for study. Five millimeter transverse segments of aorta were removed and quickly placed, for fixation, into buffered 10% formalin for 48 hours. After paraffin embedding, thin (3μ) sections were prepared. Two staining techniques were selected; one was hematoxylin and eosin used as a general oversight method; the other employed Sirius red F3B, a dye which possesses the ability to greatly enhance the birefringence of collagen fibers in a plane of polarized light (Greenberg, 1986). In this situation, they appear as brilliant orange-red bands against a black background.

Examination of the material used in this study was accomplished by means of both light and polarizing microscopy.

Results

In early life, the tunica media of the aorta is composed of a mass of well-defined smooth muscle cells. Interspersed in its long axis-are collagenous fibers which are rendered an intense red when colored by Sirius red dye and viewed by light microscopy. Under polarization, these same fibers become brilliant oranged-red bands coursing through the tunica media alongside the smooth muscle cells (Fig. 1).

Thin fibers emerge intermittently to encircle the smooth muscle cells, seemingly to bind them into a compact mass.

The collagenous fibers are evenly distributed in the tunica media of aortae from subjects under 40 years of age. Subsequently, foci of smooth muscle cells undergo

degeneration; then disappear. The collagen persists, appearing in stark contrast to areas formally occupied by smooth muscle cells.

After the fifth decade, collagenous fibers often become segregated into thickened bundles that lie near the internal and external elastic laminae (Fig. **2).** In the central portion of the media, a few thin collagenous remain fibers and an occasional band of smooth muscle.

The condensation of connective tissue into peripherally placed medial bundles occurs in both sexes, but is frequently noted to appear later in the female. As the aorta ages, there is a well-defined movement of collagenous tissue toward the periphery of the tunica media. Much of the smooth muscle in the central region appears to fade away.

Examination under polarized light of the inner connective tissue bundle, situated below the subintimal layer, reveals numerous fibers that extend upward into the subintima (Fig. **3)** where they intermingle with similar fibers already present. Connective tissue fibers proliferate beyond this level into the intima and thence into the overlying atheromatous plaques. In aortae removed from subjects aged over 70, some connective tissue fibers participating in the increasing thickness of the intimal layer find their origin within the tunica media. The subintima receives part of its complement of these fibers from the same source.

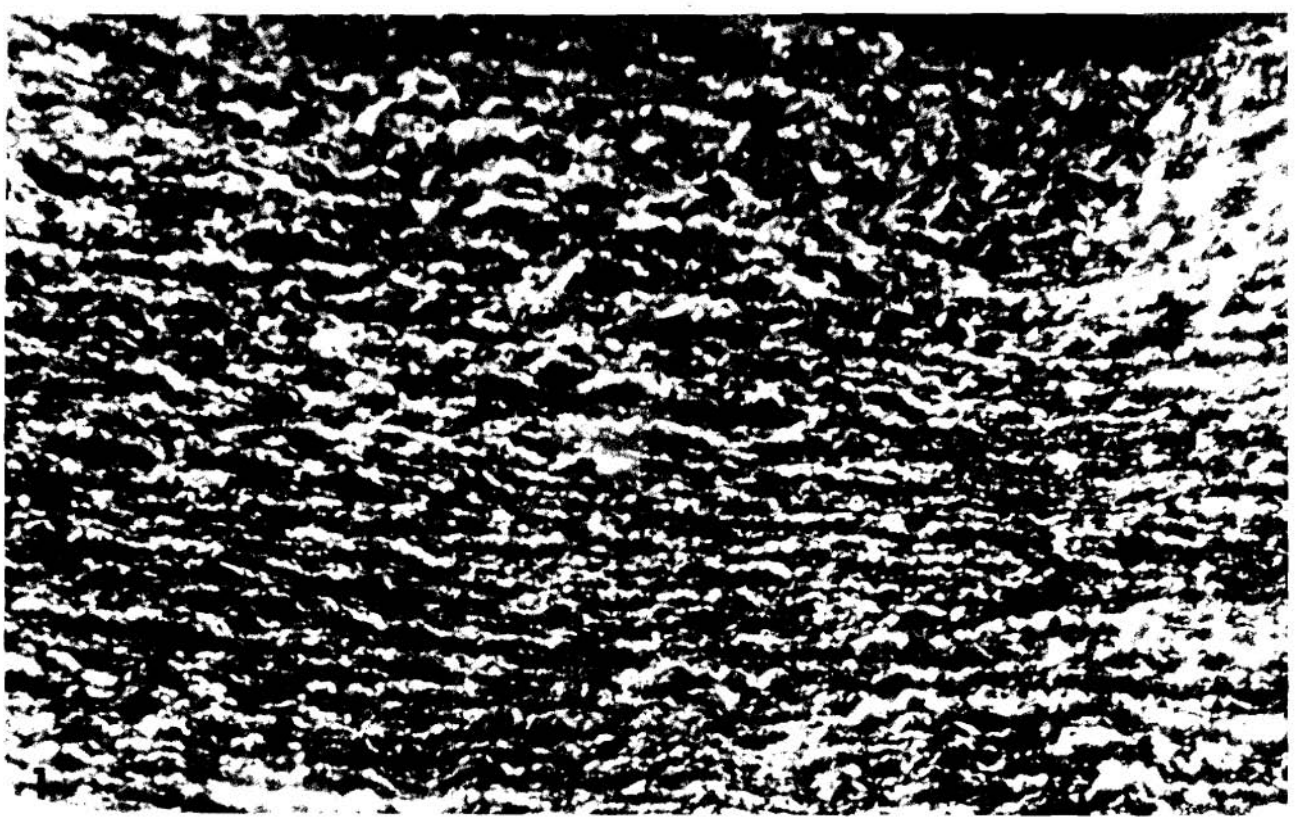

Fig. 1. Section of aorta from a boy age 10. Note the even distribution of interstitial connective tissue in the tunica media (light colored bands). x 320 Sirius red stain, polarizing microscopy.

Fig. 2. Section of aorta from a 66 year old man. Note the segregation of medial collagen fibers into two masses; one below the intimal layer; (top of picture) the other, close to the adventitia. x 160 Sirius red stain, polarizing microscopy.

Fig. 3. Section of aorta from a 64 year old man. Note the upward proliferation of collagen fibers from the periphery of the media into the subintimal layer (top of picture). x 160 Sirius red stain, polarizing microscopy.

Discussion

Both aortic interstitial connective tissue and smooth muscle are arranged in the long axis of the vessel. Examination, by polarizing microscopy, of Sirius-red stained sections of human aorta reveals that fine filaments of connective tissue course almost at right angles from the parent fibers, crossing, and encircling at intervals the smooth muscle cells in the media, binding them into a compact mass.

Close scrutiny of the junction between the tunica media and the subintima discloses the passage of thin collagenous fibers through the fenestrated internal elastic membrane into the subintimal layer where they co-mingle with similar fibers arising here (Bloom and Fawcett, 1975). Connective tissue proliferation extends beyond this level into the intima and overlying atheromatous plaques (Wolinsky and Glagov, 1969; Wisfeldt, 1986). Thus, it would appear that at least a portion of the collagenous tissue which thickens and distorts the aortic intima and the overlying atheromatous plaque arises not only in the portions of the aorta proximate to the lumen, but also within the depths of the wall. The aortic smooth muscle cells are responsible for the secretion of chondroitin sulphate and sulphate-containing proteoglycans found in the aortic ground substance (Chang et al., 1983; Wight, 1985).

Collagen continues to increase as the aorta grows older (Smith et al., 1951). Such an increase, however, may be a relative one, due, in fact, to the gradual attrition of smooth muscle cells in the aging vessel.

The division of the medial connective tissue into two peripheral zones represents a predistribution of existing fibers rather than a true quantitative increase (Roach and Burton, 1957; Abramson, 1962)

Some investigators have suggested that collagen possesses the ability to control the distensibility limits of the vascular walls. The observed peripheral distribution of these fibers within the tunica media may provide a means of

which the maximum regulatory effect is established in a vessel placed under hypertensive stress (Katzberg, 1966; Cliff, 1970).

The disparity between the thickness of the tunica media and the number of smooth muscle cells present in the walls of the aging vessels has been described in some vertebrates such as rats (Cliff, 1970), mice (Smith et al., 1951), and pigs (Nanda and Getty, 1971), as well as man (Movat et al., 1958).

Goyal (1982) has recently described, in the human aorta, a significant decrease in the number of nuclei in medial smooth muscle cells in relation to a comparable increase in the total measurable thickness of the vascular wall. Such an increase is mostly likely due to the formation of atheroma during the aging process. The loss of medial smooth muscle cells may be related to the progressive dilation of the vascular walls. This places excess pressure upon the thin-walled vasa vasorum; which, in turn, leads to a local nutritional disturbance. The peripheral segregation of medial collagen may constitute an additional factor. The subintimal mass may interfere with the ingress of nutrients from the bloodstream, further potentiating the loss of medial smooth muscle. The segregation of collagen does not antidate the loss of smooth muscle; instead, it appears in aged aortae long after the initial disappearance of muscle cells (Schutte, 1966; Schneider et al., 1977).

After the fifth decade, when atheroma has become established, collagen fibers extend into the intima, already involved by the proliferation of elastic fibers (Hall, 1976; Greenberg and Kurland, 1984), and into the overlying plaques.

Haust and her associates (1960) described the proliferation of cells ,which they identified as smooth muscle, into the intima. In their opinion, these cells were derived from endothelium that regenerated after atherosclerotic injury to the aortic wall. The Sirius-red staining technique demonstrates that these are, in reality, collagen fibers arising in the tunica media in the aged aorta.

An experimental study of the aortic wall of rabbits, fed atherosclerotic diets for three months, revealed a substantial increase in acid mucopolysaccharides, attributed to the secretion from smooth muscle cells. (Schneider et al., 1977) Further evidence in the direction was recently provided (Wight, 1985). It may be possible that part of the increased production of mucopolysaccharides in atherosclerosis may result from the secretory activity of fibroblasts.

Although the changes in the configuration of connective tissue are related to the aging process, as well as atherosclerosis, there is current evidence that chemical changes in collagen may be induced during the formation of the fatty streak (Radhakrishnamurthy et al., 1975).

Additional morphologic and biochemical studies of the aortic wall need to be undertaken before a clear picture can emerge of the relationship between smooth muscle and collagen, both in the aging process and in vascular disease (Weber, 1984).

Acknowledgements. The author greatly appreciates the technical assistance of Mrs. Jean Zygas.

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Accepted July 12, 1986