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Full Length Research Paper

Deviation of the tunica media of aorta of goat from conventional structural concept: Case for including collagen

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Knowledge of the structural organization of the aortic tunica media is important in understanding the physicomechanical properties and alterations which underpin aortic aging and pathologies such as atherosclerosis, aneurysms and aortitis. Recent studies have cast doubt on the exclusivity of the medial lamella unit and musculoelastic fascicle concepts of the aortic structure. The goat is a suitable model for studying cardiovascular disease. Therefore the structure of tunica media of its aorta was studied by transmission electron microscopy. Materials were obtained from 6 healthy adult male goats [age range 12 – 24 months]. Specimens from the mid thoracic aortic segment were fixed in 3% phosphate buffered glutaraldehyde and processed for durcupan embedding and sectioning. Ultrathin sections were stained with uranyl acetate, counterstained in lead citrate and examined by Philips 210 transmission electron microscope. Besides medial lamella units and musculoelastic fascicles in the tunica media, we observed longitudinal elastic lamellae, and cells surrounded by collagen bundles both of which were sandwiched by elastic lamellae. In some instances, cells were disposed between collagen bundles on one side, and elastic lamella on the other. These observations imply that the aortic tunica media is heterogeneous with a mosaic structure, rather than the uniform one conventionally known.

Key words: Aorta, tunica media, musculoelastic fascicles, collagen, elastic lamella.

INTRODUCTION

Knowledge of the structural organization of the aortic tunica media is important in understanding physicomechanical properties (Avolio et al., 1998; Farand et al., 2007) and alterations which underly aortic pathologies such as atherosclerosis and aneurysms (Crissman and Pakulski, 1984). Since the initial description of the medial lamella unit (MLU) of aortic structure and function by (Wolinsky and Glagov 1967) in mice, rats, guinea pig, rabbit, cat, monkey, dog, human, sheep and pig; the mammalian aortic tunica media is conventionally believed to be composed of these units, each comprising an elastic lamella and the adjacent smooth muscle cells and collagen (Awal et al., 1999; Orsi et al 2004; Gupta et al., 2011; Nowrozani, 2011; Akkila et al., 2011). In rats, rabbits and pigs, by scanning electron microscopy, the medial lamellar units could be resolved into composites of overlapping musculo-elastic fascicles (MEF) each made up of a group of commonly oriented, elongated smooth muscle cells and an encompassing array of branching similarly oriented elastic fibres (Clark and Glagov, 1985; Gasser et al., 2006, Horny et al., 2010). Successive layers of cells and their surrounding elastic fibres are separated from one another by a narrower, intervening a cellular zone containing thick wavy collagen fibre bundles. These musculoelastic fascicles are thought to constitute the structural and functional units of the aortic tunica media (Clark and Glagov, 1985; Zarins et al., 2004).

Functionally, the vascular smooth muscle cells (VSMC) are responsible for contractility, synthesis, secretion and degradation of the collagen and elastic fibres as well as ground substance (Majesky et al., 2011). The elastic fibre network maintains the distensibility such that during systole, they stretch and store energy which is released during elastic recoil to ensure a "windkessel mechanism"

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that maintains lamina flow of blood (Wegenseil et al., 2010; Wachi, 2011). Collagen, on the other hand, prevents excessive distension of the aortic wall, thus preventing rupture under excessive pressure (Cheng and Wegenseil, 2012). In this way, the three components of aortic tunica media maintain its configuration and optimum function (Popescu et al., 2013). Recent studies suggest deviations from this conventional description (Dingemans et al., 2000; Horny et al., 2010). Although, such deviations may be attributed to size (Davies et al., 1993), age (Dingemans et al., 2000) or heart disease (Niwa et al., 2001) they imply that the aortic tunica media may present structural mosaicism. Details of this are still not elucidated.

The goat is a suitable model for studying cardiovascular disease (Lemson et al., 1999; Zheng et al., 2000) because the structure of some of its vessels, and its physiological cardiovascular parameters ressemble those of human (Manrique et al., 1977; Garcia et al., 1995; Prassinos et al., 2005). This study, therefore aimed at describing the ultrastructural organization of tunica media in the aorta of young adult goats.

MATERIALS AND METHODS

Materials were obtained from 6 adult healthy male goats only. This was to avoid potential gender related differences. The animals were young adults age range (12 – 24) certified to be healthy by veterinarians in whose arteries there were no age changes. The animals were euthanized with overdose of sodium pentabarbitone 20mg/ml injected intraperitoneally and fixed by perfusion using 3% buffered glutaraldehyde solution. Specimens were obtained from mid thoracic region of the aorta, were routinely prepared for transmission electron microscopy. They were immersed in 3% phosphate buffered glutaraldehyde solution for 72 hours for further fixation, and subsequently post fixed with 1% phosphate buffered osmium tetroxide solution for 48 hours.

The post-fixed specimens were rinsed in sodium phosphate buffer for 15 minutes then dehydrated in ethanol. The sections were cleared in propylene oxide for 30 minutes, and subsequently infiltrated in catalyst free durcupan mixture I. They were then embedded in 100% durcupan with catalyst, and polymerized in an oven at 600c, for 48 hours. The blocks were trimmed and ultrathin sections made with Reichert ultramicrotome©. These sections were collected on 200 mesh copper grids, stained with uranyl acetate, counterstained with lead citrate, and examined by EM 201 Phillips© transmission electron microscope.

RESULTS

The tunica media was the thickest layer, extending between the internal and external elastic laminae. It was elastic in nature. In most places, it was made up of regular elastic lamellae between which were collagen and smooth muscle cells (Figure 1A) forming medial lamellar units (MLU). Some of the elastic lamellae branched. A number of the branching sites were intimately associated with collagen bundles which appeared to insert onto the elastic fibres (Figure 1A). In most instances, the units could be resolved into musculoelastic fascicles (MEF) comprising composites of smooth muscle cells, elastic lamellae and adjacent collagen bundles (Figure 1B). A few elastic lamellae, displayed discontinuities, which contained cell processes, with cell processes appearing to anchor onto the elastic lamellae. Other gaps were bridged by collagen fibres which also seemed attached onto the elastic fibres (Figure 1C). In other instances, however, elastic lamellae run longitudinally in the interval between elastic lamellae. Collagen bundles frequently attached to such lamellae. (Figure 1D). Frequently, elastic lamellae ran in parallel with collagen fibres with some collagen attaching on the sides of the lamella or end-on (Figure 1E).

Deviations from the MLU and MEF concepts were consistently observed in the luminal zone of tunica media in all the six animals studied. Similar deviations were noted in other regions of the thoracic aorta. The most notable deviations where either the cells were surrounded by large collagen bundles running perpendicular to the elastic lamellae with the latter sandwiching both (Figure 2A), or where a cell was associated with collagen bundle on one side, and elastic lamella on the other.

The elastic lamellae appeared to bend to accommodate the cell whose processes would ran parallel to the circumferentially oriented elastic lamellae and collagen bundles (Figure 2B). Frequently, an elastic lamella and a collagen bundle ran perpendicular to each other with some collagen fibres apparently attaching onto the elastic fibres. Collagen bundles from both sides of same lamellae would converge to form a thicker bundle (Figure 2C). In many instances, collagen bundles and elastic lamellae/fibres ran parallel and alternated with each other. Again, in cases where the elastic lamella branched, collagen was seen filling the space between the branches (Figure 2D).

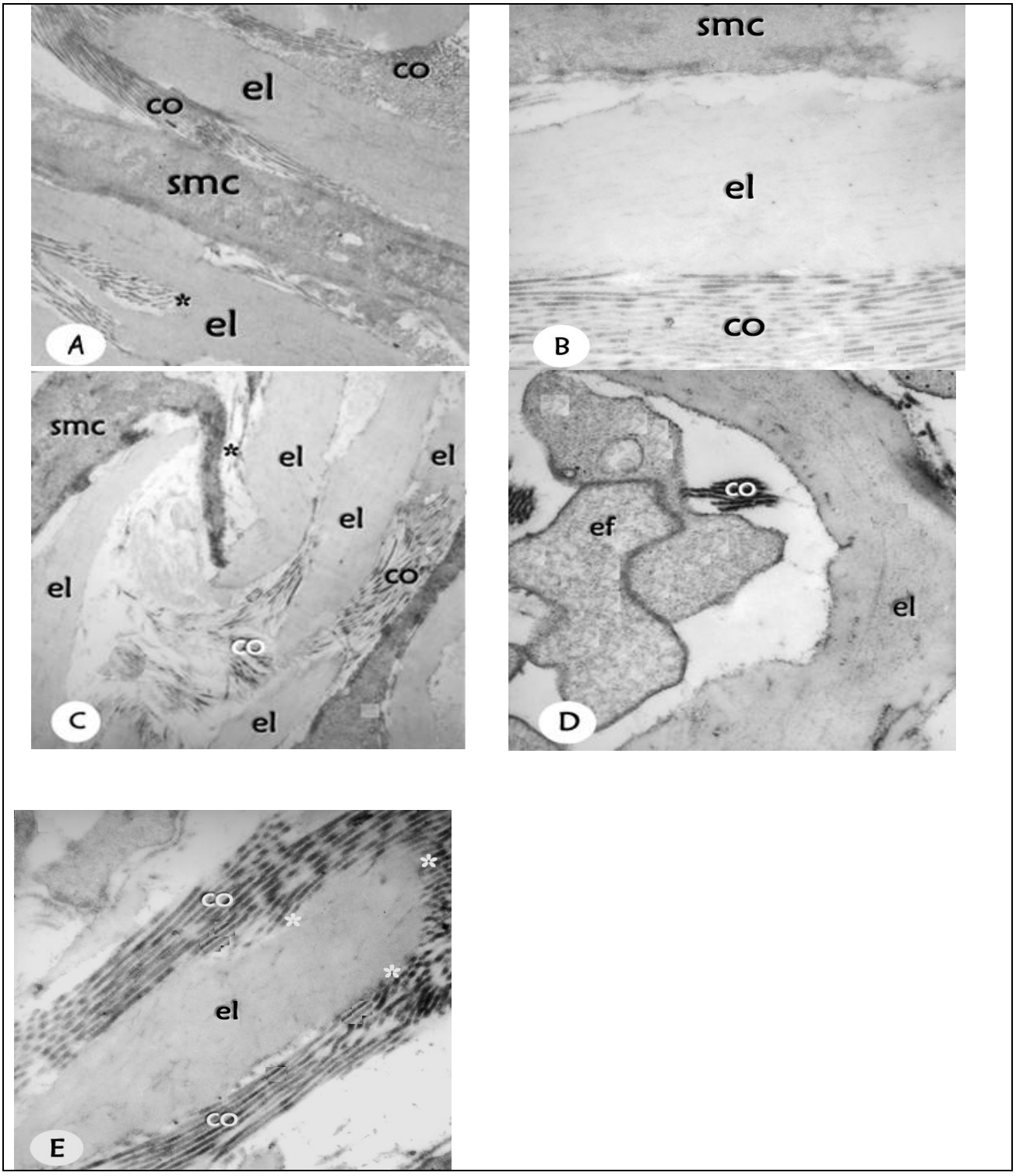
Figure 1A-E: Electron micrograph of showing organization of tunica media of goat aorta.

A: Smooth muscle cell (smc) and adjacent elastic lamellae (el). Observe paracellular collagen (co), and branching of elastic lamella (star) x 8,760

B: Three alternating components that form the musculo elastic fascicle (MEF), namely smooth muscle cell (smc), elastic lamellae (el) and collagen bundle (co) x 63,400.

C: Discontinuity in an elastic lamella (el). Note a process of a smooth muscle cell (smc) in the gap (star) and collagen (co) bridging another discontinuity between elastic lamellae. x8,760.

Figure 1.



D: Longitudinal elastic fibres (ef), and the elastic lamellar (el). Note collagen fibres (co) physically linking onto the elastic fibres. x27,800.

E: An elastic lamella (el) surrounded by collagen fibres (co). Note that the most adjacent collagen fibres insert onto the elastic lamella (stars). x63,400.

Figure 2.

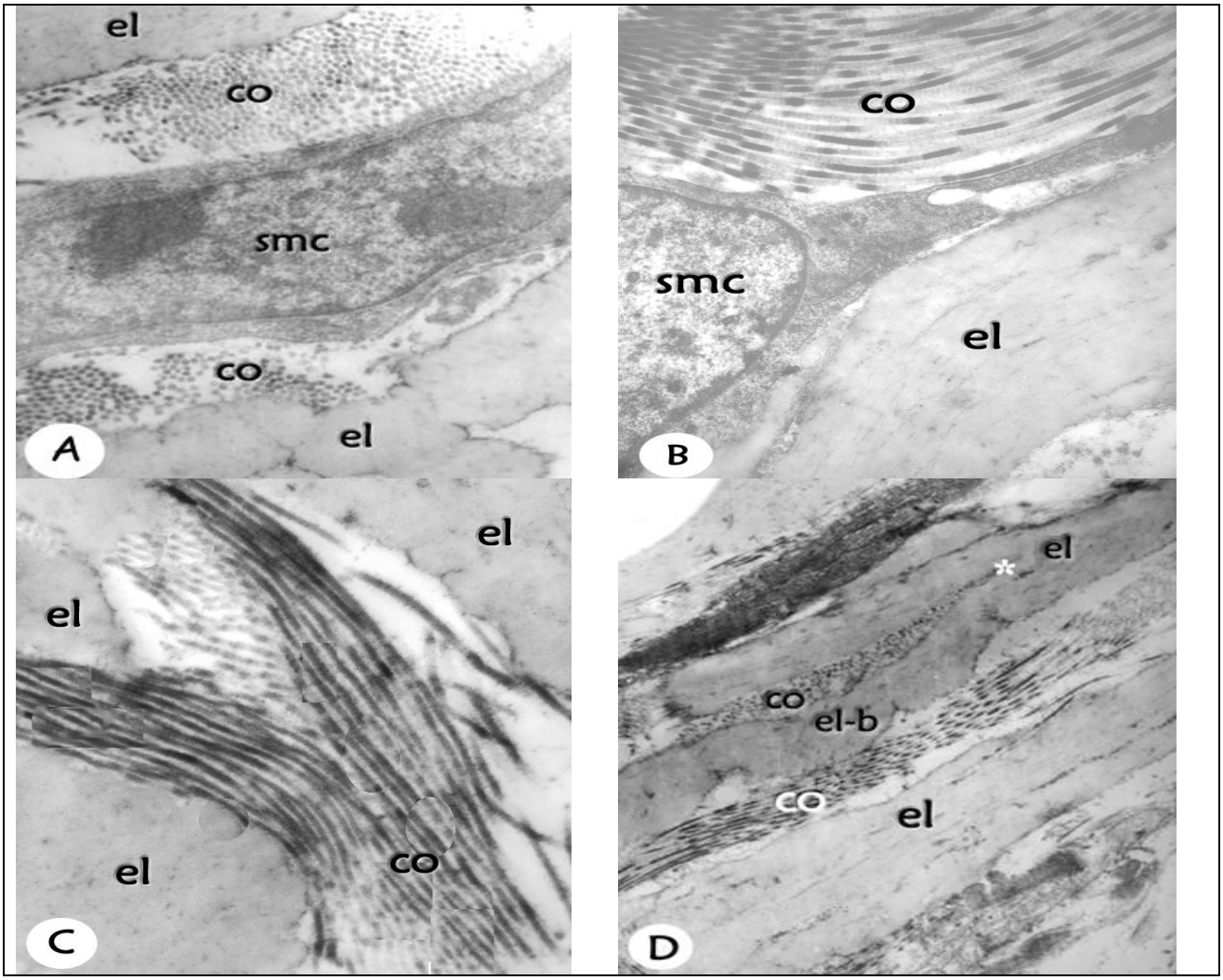


Figure 2A-D: Electron micrograph of tunica media of goat aorta showing deviation from the Musculo Elastic Fascicle concept

A: A smooth muscle cell (smc) surrounded by bundles of collagen (co). Note how both the cell and the surrounding collagen are sandwiched by elastic lamellae (el). x63,400.

B: A smooth muscle cell (smc) surrounded by collagen bundle (co) on one side, and elastic lamella (el) on the other. x63,400.

C: Collagen bundle (co) running perpendicular to elastic lamellae (el). x63,400.

D: A branch of elastic lamella (el-b) sandwiched by bundles of collagen (co).

Note the collagen fibres running in different directions with some parallel, and alternating with elastic lamellae, the branching point (star) of some of the elastic lamellae (el). x8,760.

DISCUSSION

Many parts of the tunica media of the goat aorta comprise Medial Lamellae Units (MLU) as described in rats (Clark and Glagov, 1985), human (Dingemans et al., 2000) sheep, dogs and cats (Song and Roach, 1985); pigs (Snowhill et al., 2004) and rabbits (Farand, 2007). These workers proposed that the elastic lamellae and fibres provide sites of attachment for smooth muscle cells and uniformly distribute the forces across the aortic wall during systole. The concentric elastic lamellae are also believed to constitute a windkessel mechanism that helps to propel blood to the periphery during diastole, thus maintaining laminar flow (Orsi et al., 2004; Snowhill et al., 2004). In addition, the lamellae are thought to bear the circumferential forces while the branches and other fibres distribute the forces uniformly (Dingemans et al., 2000; Tonar et al., 2003). It is therefore possible that the elastic

fibre organization ensures mechanical strength, force distribution and the windkessel mechanism.

In some parts of the tunica media, the smooth muscle cells or their processes alternate in harmony with elastic lamellae and the two are then sandwiched by collagen, to conform with the Musculo Elastic Fascicle (MEF) unit proposed by (Clark and Glagov, 1985) in rats, rabbits and pigs and subsequently supported in dogs (Nowrozani et al., 2011). They, however, appear at variance with those of (Davies, 1993) in the mouse and in the human (Dingemans et al., 2000). The former failed to recognize the unit proposed by (Clark and Glagov 1985) and suggested that this organization may be a feature of the aorta of larger animals. Dingemans et al., (2000), on the other hand, considered that MEFs may show age variation. It is possible that both Davies (1993); Dingemans et al. (2000), examined different areas of the aortic tunica media as (Dingemans et al., 2000) suggested, many observations regarding the organization of the extracellular matrix in the aorta are not controversial per because they are derived from randomly sampled specimens.

The interlamellar spaces of the tunica media contain not just branches but definite elastic fibres running in different directions, similar to those in human aortic media (Jiang et al., 1995; Dingemans et al., 2000). The interlamellar connections to the main lamellae may also determine distensibility properties of the aorta, (Nakashima and Sueishi, 1992). The structural orientation throughout the wall, and the complex interlocked structure described in present study may be important in maintaining the mechanical properties of the aorta (Avolio et al., 1998).

Observations of the present study further reveal that some of the elastic lamellae are arranged longitudinally and obliquely. It has been suggested that elastic lamellae and fibres, which form a complex meshwork, strengthen the aortic wall in such a manner that it can withstand multidirectional stresses imposed onto it by ventricular systole (Gosline and Shadwick 1996; Shadwick, 1999). Pertinent to this suggestion is the recent proposal that the array of tunica media elastic fibres perpendicular to luminal pulsatile flow gives additional strength to the elastic lamellae in supporting circumferential stress, while those arranged parallel to luminal flow are best adapted to sustaining longitudinal stress (Farand et al., 2007). Accordingly, the final three-dimensional structure of elastic fibres, as well as their interaction with other elements of the extracellular matrix and smooth muscles, ultimately determines the mechanical properties of the aorta.

A novel finding of the current study is that in most cases the smooth muscle cells of the goat aortic tunica media are immediately surrounded by collagen which separates them from elastic lamellae. This observation is at variance with the description of (Clark and Glagov 1985) among rabbits, rats and pigs, subsequently supported by (Gasser et al., 2006; Horny et al., 2010) in which the functional unit called the Musculo Elastic Fascicle (MEF) comprised smooth cells and elastic lamellae, which are then bracketed by collagen. They are also at variance with previous reports on the human thoracic aortic tunica media in which a preferential localization of collagen fibrils immediately adjacent to the smooth muscle surface was not observed (Dingemans et al., 2000). We propose, in support of Snowhill et al., (2004), revision of the lamellar unit to fit collagen. Although (Snowhill et al 2004) study shows the same cellular layers bounded by elastin, these workers suggested that the lamellar unit should consist of collagen and smooth muscle bounded by a variable concentration of elastic fibres. The new unit would be smooth muscle cells bounded by collagen, in a 1:1 volume fraction ratio and then bounded by two elastic laminae one on the luminal side and the other on the abluminal side. Observations of the present study suggest that collagen fibres play a more important role in the structure and function of the aorta than previously appreciated, and should be included in the definition of the structural unit of the aortic tunica media. Pertinent to this suggestion is the ascertion that the three components – smooth muscle cells, collagen and elastin maintain the aortic configuration (Popescu et al., 2013).

CONCLUSION

The aortic media is heterogeneous with a mosaic structure comprising various structural combinations of arrangements and interactions of cells and extracellular matrix rather than the uniform one conventionally known. This mosaism in which collagen plays a more important role is responsible for the dynamism, distensibility and high physicomechanical strength of the vessel. This enables it to withstand the force of systole and also provide the elastic buffer capacity and allow requisite laminar flow of blood. The conventional MLU and MEF concepts should be revised to accommodate collagen.

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CONFLICT OF INTEREST: none

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