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TRANSMEDIAL ZONATION AND REGIONAL VARIATIONS IN TUNICA MEDIA OF AORTA IN SHEEP (*Ovis aries*)

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ABSTRACT

The sheep is a valuable model for cardiovascular studies and its aorta is frequently afflicted by atherosclerosis and aneurysms. The structure of sheep aorta is, however, only seldom reported. This study, therefore, aimed at describing the microscopic organization of the tunica media of the aorta in sheep. Specimens obtained from the aortae of six healthy young adult sheep were fixed in 10% formaldehyde solution and routinely processed for paraffin embedding and sectioning. Five micron sections were stained with Weigert's Resorcin Fuchsin/Van Gieson and examined with a light microscope. Micrographs were taken with a high resolution digital camera. The tunica media in the ascending, arch and thoracic regions comprised two zones namely a luminal elastic zone with continuous uniform elastic lamellae and adventitial musculo-elastic one where there were muscle islands frequently interrupting the elastic lamellae. The proportion of musculo-elastic zone with the muscle islands declined caudally. In the abdominal region, however, the tunica media comprised regular continuous concentric elastic lamellae. These results show that the aorta in sheep displays regional variations characterized by transmedial zonation of the thoracic segments into an elastic luminal and musculo-elastic adventitial zones. These differences may underpin the regional differences in physicomechanical properties and vulnerability of the aorta to diseases such as atherosclerosis and aneurysm.

Keywords: Tunica media, aorta, muscle islands, sheep.

INTRODUCTION

The structure of the aorta influences its physicomechanical properties, functions and vulnerability to diseases such as atherosclerosis and aneurysm (Sokolis et al., 2006; O'Connel et al., 2008). For a long time, the mammalian aorta has been believed to comprise conventional uniform continuous medial lamella units (Wolinsky and Glagov, 1967). Our recent studies indicate that the structure of the aorta, in goat for example, deviates from this model (Ogeng'o et al., 2009, 2010, 2014) suggesting that in using the animal models for cardiovascular research, each animal model must be considered on its own merit. Sheep develop spontaneous atherosclerosis similar in regional distribution to human beings (Gupta and Nagpal, 1979) and are being used extensively in research on aortic disease (White et al., 1998; Boudghene et al., 1998) and experimental cardiovascular surgery (Shum – Tim et al., 1999; Elkins et al., 2001; Dohmen et al., 2001).

Further, because of its similarity to humans in important respects, the sheep are now common models for translational research in cardiovascular surgery (Louis et al., 2014). Inspite of this high research premium, the structure of the aorta in sheep is only seldom reported. This study, therefore, aimed at describing the organization of the tunica media of aorta in sheep.

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MATERIALS AND METHODS

Materials for this study were obtained from 6 healthy young adult male sheep (*Ovis aries*) [Age range 6 – 12 months]. The sheep were purchased from local farmers in Nairobi and confirmed to be healthy by a veterinary doctor. The animals were euthanized by overdose 5% sodium pentabarbital and perfused with 10% formaldehyde solution. Two millimeter long aortic specimens were obtained from the middle of ascending aorta, arch and every segment of the thoracic aorta down to the level of thoracic diaphragm at T13 and from the pre-renal and post-renal segments of the abdominal aorta. The specimens were further fixed by immersion in formaldehyde solution for 72 hours, rinsed, dehydrated in ethanol, cleared in cedar wood oil, and infiltrated in paraffin wax at 60^0c for 12 hours. Five micron thick transverse sections were made and stained with Weigert's Resorcin Fuchsin/Van Gieson. They were examined with a light microscope and photographed with a high resolution digital camera. The results are presented in digital micrographs.

RESULTS

The tunica media of the aorta of the sheep was predominantly elastic. It, however, varied with region. In the ascending, arch and thoracic segments, it was divisible into a luminal and an adventitial zone. The luminal zone comprised continuous concentric elastic lamellae, while in the adventitial zone, the elastic lamellae were interrupted by definite muscle islands (Fig 1 A - C). The muscle islands were most prominent in the ascending aorta where they occupied about 75% of the tunica media [Fig 1A]. The proportion of the muscle islands declined caudally such that it was about 70% in the arch [Fig 1B]; 66% in the upper thoracic, 25% in the mid thoracic [Fig 1C] and less than 10% in the distal thoracic region. At the level of thoracic diaphragm, the muscle islands were scanty and confined to the immediate sub adventitial region [Fig 1D]. In the abdominal segment, distinct from the thoracic ones, the entire tunica media comprised uniform concentric elastic lamellae without any evidence of zonation or interruption [Fig 1E].

DISCUSSION

Observations of the present study reveal a transmedial zonation of the tunica media of the proximal segments characterized by presence of muscle islands in the adventitial zone. This is at variance with the widely accepted conventional structure described by Wolinsky and Glagov (1967) and Glagov and Clark (1979, 1985) in the general mammalian aorta. It is also at variance with the description of Van Baardwijk and Roach (1983). These differences may be due to the site or region studied, but nonetheless reveal that the structure of the aorta is not uniform across mammals.

The presence of muscle islands is concordant with the findings in aorta of cow (Knieriem, 1967), fetal lambs (Fukuda et al., 1984), goat (Ogeng'o et al., 2009; 2010), giraffe carotid arteries (Kimani and Opole, 1991) and subclavian arteries in lamb (Miclaus et al., 2015). Vascular smooth muscle cells in great arteries keep vasomotor tone under stable conditions through complex regulating mechanisms. Indeed, local activation of the vascular smooth muscle in conduit arteries produce an increase of its elasticity and viscocity even when pressure remains constant (Salvucci et al., 2007) and play an important role when variations of pressure and flow occur, modulating the degree of deformation and shear rate, which ultimately

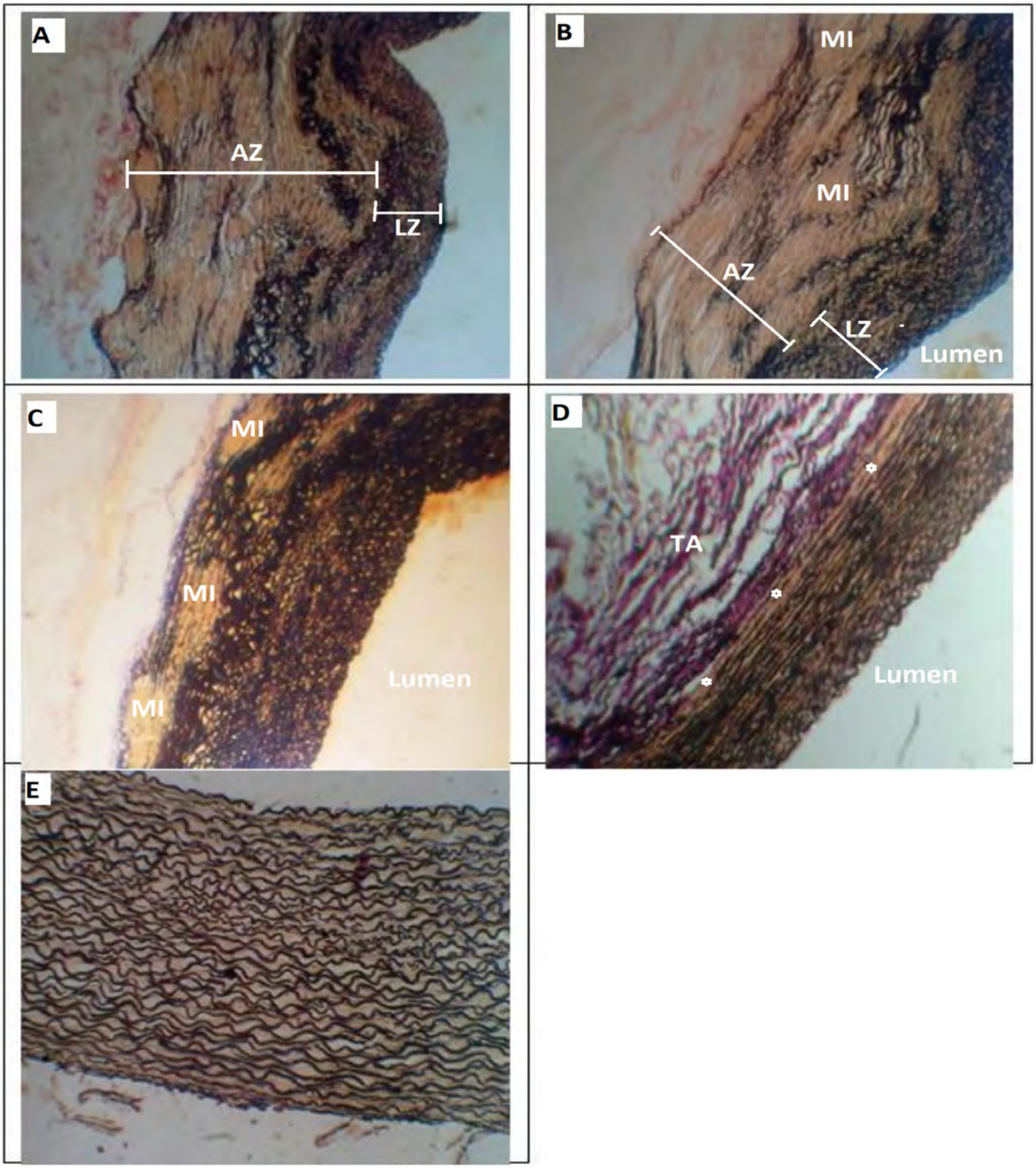

Figure 1 A – E: Microscopic appearance of the aorta of sheep (Weigert's Resorcin Fuchsin/Van Gieson stain: X100). AZ = Adventitial zone; LZ = Luminal zone; MI = Muscle island; TA = Tunica adventitia. **A:** Ascending Aorta: The muscular AZ is musculoelastic with only a narrow elastic LZ. **B**: Arch of aorta with musculoelastic zone occupying about 70%. Note the elastic fibres joining some muscle islands (MI). **C**: Mid-thoracic aorta with receding muscular zone occupying about 25% of the tunica media. **D**: Distal thoracic aorta at the vertebral level T_{13}. Note the scanty thin muscle strips (*) towards the tunica adventitia. **E**: Abdominal aorta with continuous elastic lamellae in the entire tunica media.

determine the mechanical response of the vascular wall. According to the type of response, they can be described as pressure dependent myogenic responses (Davies and Hill, 1999; Schubert and Mulvany, 1999; Frides et al., 2001) or flow dependent endothelium mediated responses (Davies et al., 2003). These mechanisms are described as short term adaptive phenomena whose purpose is to maintain, inside the arterial wall, a circumferential stress at a normal and uniform level (Glagov et al., 1992). Mechanical properties and responses of the aorta have previously only been considered in light of the elastin and collagen constituents, totally disregarding smooth muscle cells (Sokolis et al., 2006). Observations of the current study suggest that smooth muscle is involved in determination of mechanical properties of aortic wall.

The transmedial zonation may be related to the distribution of strain. Studies have shown that the luminal and adventitial zones of the tunica media experience different levels of strain in different circumstances (Vaishnav & Vassoughi, 1987). Further, residual circumferential stress may contribute to vascular remodeling (Fung, 1991). The structural organization of the elastic components of the wall probably affect the maximum stress at the lumen for a given blood pressure (Avolio et al., 1998). Because of the smooth muscle property of myogenic response (Meinger and Davies, 1992), the muscle islands have been ascribed a contractile function which facilitates blood flow, augmenting the windkessel mechanism (Ogeng'o et al., 2009). In subclavian arteries, the islands are considered important in regulating blood flow to the target regions (Miclaus et al., 2015). Secondly, the smooth muscle cell activation is important for maintaining local and global buffering functions in the cardiovascular system (Bia et al., 2003).
It is plaucible therefore that the increased contractile muscle mass in the adventitial zone of the tunica media described in the present study, by myogenic response (Johanson, 1989; Civelek et. al., 2002, Fanchaouy et. al., 2007), increases the mechanical strength of the aortic wall. In this regard previous studies have demonstrated that active vascular muscle does resist distension upto 150 – 250 mmHg (Dobrin, 1984). Further, aortic smooth muscle may change its contractile state through myogenic response to keep intramural strain distribution uniform against temporary changes in blood pressure and thus maintain mechanical homeostasis in the aortic wall (Matsumoto et. al., 1996). A higher mechanical strength may be especially required at higher strains where the behaviour of the vessel wall has been shown to be consistent with an in series arrangement of collagen and smooth muscle (Silver et. al., 2003). A pertinent observation of the present study is that in the adventitial zone of the tunica media where the strain may exceed that in the luminal zone (Guo et. al., 2005) some of the muscle islands are in series with the matrix.

The regional differences observed are similar to those described in the goat aorta (Ogeng'o et al., 2010). We postulated that these differences were related to the profile of strain. Accordingly, it is plaucible that similar mechanisms operate in the sheep. The muscle islands may constitute a strengthening mechanism for the proximal segments of the aorta which are subjected to direct ventricular thrust and also constitute auxillary blood propelling mechanism (Ogeng'o et al., 2009). It is probable that by the time blood reaches the abdominal aorta, the pressure can be borne by the conventional MLU structure and blood flow by windkessel mechanism, without need for augmentation.

CONCLUSION

The tunica media of the aorta in sheep displays zonal and regional differences characterized by muscle islands in the adventitial zone of the proximal aortic segments. These differences may underpin the regional predisposition of abdominal aorta to atherosclerosis and should be borne in mind in the design of aortic substitutes and studies of aortic disease.

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CONFLICT OF INTEREST: There is no conflict of interest

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