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NON MUSCLE CELLS IN THE TUNICA MEDIA OF THE AORTA

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SUMMARY

Knowledge of cellular composition of aortic tunica media is important to improve understanding of aortic pathology. The aorta of 6 healthy male goats was studied by electron microscopy to elucidate cell types within the tunica media. Glutaraldehyde fixed specimens were processed for durcupan embedding and sectioning, stained with uranyl acetate, counterstained with lead citrate and ultrathin sections examined at high magnification. Two non muscle cells were observed, one resembling fibroblasts and the other with features of macrophages. It is concluded that these cells are normal constituents of the aortic media, involved in synthesis of extracellular matrix and immunosurveillance respectively. Their involvement in repair and disease process needs further investigation.

Keywords: tunica media, aorta, fibroblast, macrophage.

INTRODUCTION

Cellular composition of the tunica media is important in maintaining the structural integrity (Siedel, 1997; Bia et al., 2003) and physicomechanical properties of the aortic wall (Bia et al., 2003; Van Leeuwen, 2003). Most studies report the tunica media to comprise only the smooth muscle cell and its variants (Vilaschi et al., 1994; Bochaton – Pillat et al., 1996; Frid et al., 1997). The morphological data in favour of this cellular homogeneity has, however, been challenged by immunohistochemical and cloning data indicating cellular heterogeneity (Seidel, 1997; Sainz et al., 2006). Some of these cells have been implicated in arterial disease and repair (Seidel 1997; Tonar et al., 2010). Knowledge of the cell types found in normal aortic wall will improve understanding of the aortic diseases such as of atherosclerosis and aneurysms. The goat is a suitable model for studying cardiovascular disease (Lemson et al., 1999; Zheng et al., 2000). The structure of its aorta was, therefore, studied to elucidate the cellular composition of the tunica media.

MATERIALS AND METHODS

Materials for this study were obtained from 6 healthy male goats (*Capra hircus*) aged 6 to 24 months. The animals were euthanized with overdose of sodium pentabarbitone and perfused with buffered 3% glutaraldehyde. 2mm^3 sections were postfixed in osmium tetroxide. The post-fixed specimens were rinsed in sodium phosphate buffer for 15 minutes then dehydrated by passing through increasing concentrations of ethanol (50%; 60%; 70%; 80%; 90%; 95% and 100%) for 30 minutes each, and twice for 1 hour each in absolute ethanol. The sections were then cleared in propylene oxide for 30 minutes. Subsequently, the sections were infiltrated in catalyst free durcupan mixture I as follows: propylene oxide: durcupan 3:1 – 30

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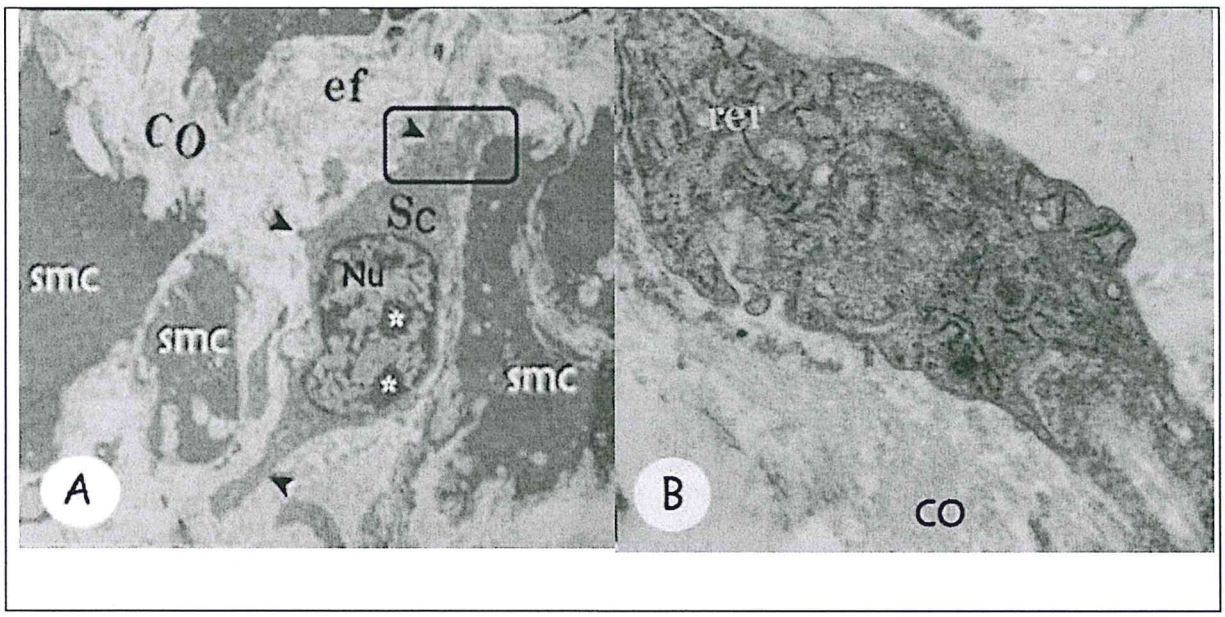
minutes; propylene oxide: durcupan 1:1 – 30 minutes; propylene oxide: durcupan 1:3 – 30 minutes and absolute durcupan at 60^0C in oven for one hour. The sections were then embedded in 100% durcupan with catalyst, and polymerized in an oven at 60^0c, for 48 hours. Ultrathin sections made with *Reichert ultramicrotome*© were collected on 200 mesh copper grids, stained with uranyl acetate, counterstained with lead citrate (Glauert, 1965), and examined by *EM 201 Phillips*© electron microscope.

RESULTS

In the muscle islands of the adventitial zone in the tunica media, the interfascicular space contained smaller cells with large euchromatic nuclei and prominent nucleoli (Fig 1A). These cells were characterized by extensive cytoplasmic processes which ran between the matrix components. The cytoplasm in the perinuclear region, and within the processes shows prominent rough endoplasmic reticulum and mitochondria, but scarce microfilaments (Figure 1B). These cells also lacked remnants of a basal lamina, and resembled fibroblasts. Similar fibroblast-like, but much more slender and elongated, cells were found between the elastic lamellae joining the muscle islands (Figure 1C). A second type of cells was observed in some folds of elastic lamellae. Such cells were characterized by a kidney shaped nucleus, irregular cell surface and numerous lysosome-like structures (Figure 1D).



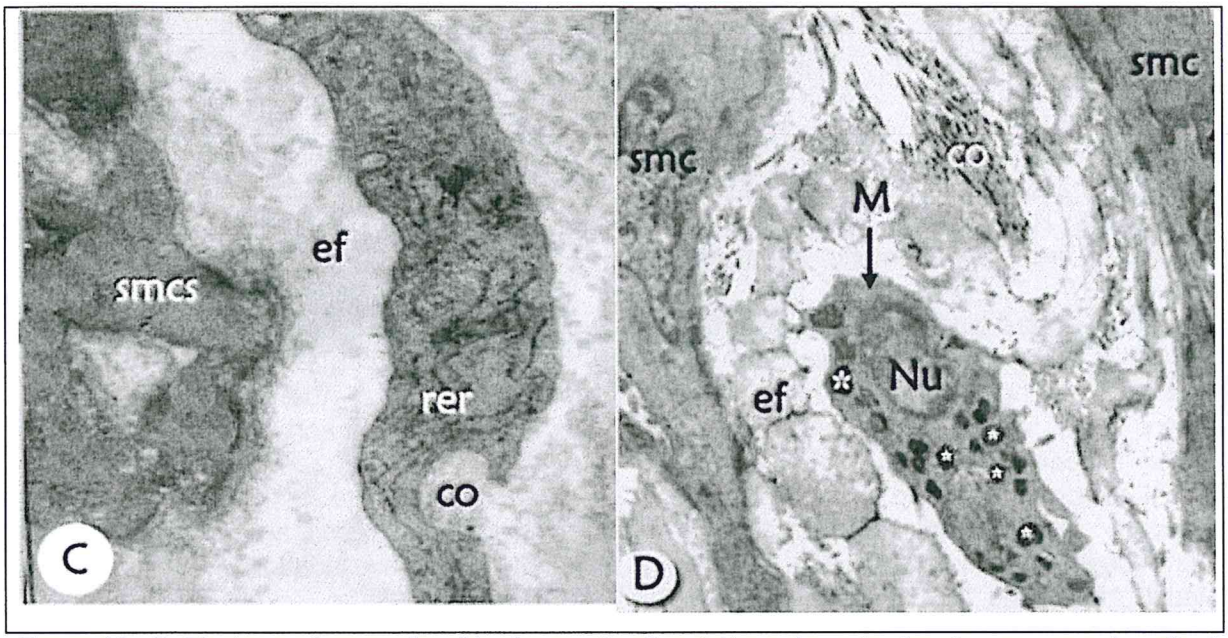


Figure 1A –D: Electron micrographs of tunica media of goat thoracic aorta showing non-muscle cells. A: A synthetic cell (sc) bearing cytoplasmic extensions (arrowheads), and euchromatic nucleus (Nu) with two nucleoli (stars). Note the surrounding smooth muscle cells (smc), elastic (ef) and collagen (co) fibres. x8,760. B: Part of the synthetic cell in figure 1A. Note the abundance of rough endoplasmic reticulum (rer), and also collagen (co) in the neighbourhood. x63,400. C: Part of an interlamellar elongated cell with extensive rough endoplasmic reticulum (rer), and absent basal lamina. Note elastic fibres (ef), collagen (co) fibres and the smooth muscle cell (smc) in the neighbourhood. x63,400. D: A macrophage-like (M) cell with an irregular outline, kidney shaped nucleus (Nu) and lysosome-like structures (stars). Note its proximity to elastic fibre (ef), collagen (co) and smooth muscle cells (smc). x 8,760.

DISCUSSION

The tunica media of the goat aorta contains, apart from smooth muscle cells of variable sizes, fibroblast-like cells, characterised by cytoplasmic processes, abundant rough endoplasmic reticulum, euchromatic nucleus, prominent nucleolus, and absence of myofilaments and basal lamina. The demonstration of these cells is at variance with the generally accepted view that the arterial tunica media consists of only the smooth muscle cells and their phenotypic variants (Wissler, 1968; Villaschi *et al.*, 1994; Bochaton-Pillat *et al.*, 1996; Frid *et al.*, 1997; Nowrozani, 2011). Similar cells have been reported in the aortic tunica media of the dog by Geer *et al.,* (1961) and chicken by Moss and Bendit (1970), who called them interlamellar connective tissue cells, and postulated that they were involved in the synthesis of extracellular matrix. It is plausible that similar cells differentiated within the tunica media to specifically serve the function of matrix synthesis and secretion so that they relieve the smooth muscle cells of this function. In this way, the smooth muscle cells, free of synthetic role, concentrate on contractility.

The other cell type in the tunica media is characterized by large dented nuclei, vacuoles with dark material, cytoplasmic extensions and lysosome-like structures. These features are usually associated with macrophages. The presence of these "macrophage-like" cells supports reports that macrophages, and vascular dendritic cells, exist in the tunica media of the healthy rabbit, and human aortae (Hineck and Rosnowski 1975; Hineck and Konsinski, 1975; Krupa *et al.*, 2002; 2004).

Both fibroblast – like cells and macrophages are usually associated with atherosclerosis (Toda, 1988; Halloran et al., 1997; Sako et al., 2003) and asymptomatic aneurysms (Tonar, 2010). The presence of these cells in healthy aortae supports the view that such cells, are normal constituents of the aortic wall. Their demonstration, in the present study, in young free-ranging goats further supports the view that they are normal components of the aorta, which are important in synthesis of extracellular matrix fibers namely elastin and collagen (Siedel, 1997) and in maintaining immune surveillance in the aortic wall by phagocytosis and antigen presentation (Weyand *et al.*, 2005; Galkina *et al.*, 2006).

In conclusion, two non-muscle cells were observed, one resembling fibroblasts and the other with features of macrophages. It is concluded that these cells are normal constituents of the aortic media, involved in synthesis of extracellular matrix and Immunosurveillance respectively. Their involvement in repair and disease process needs further investigation.

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CONFLICT OF INEREST: None

REFERENCES

1. Bia D, Armentano RL, Grignola J, Craiem D, Zocalo YA, Gines FF, Levenson J (2003). The vascular smooth muscle of great arteries: Local control site of arterial buffering function? Rev Esp Cardiol 56: 1202 – 1209.
2. Bochaton-Pillat ML, Ropraz P, Gabbiani F, Gabbiani G (1996). Phenotypic heterogeneity of rat arterial smooth muscle cell clones. Arteriosclerosis, Thrombosis and Vascular Biology 16:815-820.
3. Frid MG, Dempsey E, Durmowicz A, Stenmark K (1997). SMC heterogeneity in pulmonary and systemic vessels: importance in vascular disease. Arteriosclerosis. Thrombosis and Vascular Biology 17:1203-1209.
4. Galkina E, Kadl A, Sanders J, Karughese D, Sarembok IJ, Ley K (2006). Lymphocyte recruitment into the aortic wall before and during development of atherosclerosis in partially L-selectin dependent. The Journal of Experimental Medicine 203: 1273 – 1282.
5. Geer JC, McGill HC Jr, Strong JP (1961). Fine structure of human atherosclerotic lesions. American Journal of Pathology 38: 263-288.
6. Glauert AM (1965). Techniques for electron microscopy. D. Kay (Ed). Blackwell Scientific Publications. Oxford, 2nd Edition: 166-310.
7. Halloran BG, Grange JJ, So BJ, Baxter BT, Nebraska O (1997). Macrophage products inhibit human aortic smooth muscle cell proliferation and after 1 ∞ (I) procollagen expression. Ann Vasc Surg 11: 80 – 84.
8. Hineck A, Konsinski M (1975). Electron microscopic studies on cells isolated from aorta of newborn and adult rabbits. Polish Medicine, Science and Histology Bulletin 15: 9 – 22.
9. Krupa WM, Dewan M, Jeon MS (2002). Trapping of misdirected dendritic cells in the granulomatous lesions of giant arteritis. American Journal of Pathology 161:1815-1823.
10. Leeuwen MY (2003). Effect of decellularization on mechanical properties of porcine left carotid arteries. BMT 03 – 12 TUE: 1 – 20.

11. Lemson MS, Daemen MJ, Kitshaar PJ, Tordoir JH (1999). A new animal model to study intimal hyperplasia in Av fistula. Journal of Surgical Research 85:51-58.
12. Moss NS, Benditt EP (1970). Spontaneous and experimentally induced arterial lesions, part 1 (an ultrastructural survey of the normal chicken aorta). Laboratory Investigation 22:166.
13. Nowrozani FR (2011). Comparison of abdominal aorta and renal artery in the neonatal male dog. J Animal Vet Adv 10 (17): 2278 – 2281.
14. Sainz J, Al Haj ZA, Caligiuri G, Demerens C, Urbain D, Lemitre M, Lafont A (2006). Isolation of "side population" progenitor cells from healthy arteries of adult mice. Arterioscler Thromb Vasc Biol; 26: 281 – 286.
15. Sako T, Uchida E, Kagawa Y, Hirayama K, Nakade T, Taniyama H (2003). Immunohistochemical detection of apoliprotein A – 1 and B – 100in carine atherosclerotic lesions. Vet Pathol40: 328 – 331.
16. Siedel CL (1997). Cellular heterogeneity of the vascular tunica media: Implications for vessel wall repair. Arterioscler Thromb Vasc Biol17: 1868 – 1871.
17. Toda T (1988). Immunohistochemical and ultrastructural study of aortic lesions in fat – fed quails. Jikken Dobutsu Exp Animals37 (2): 179 – 185.
18. Tonar Z, Witter K, Krizkova V, Eberlova L, Kocova J, Molacek J, Houdek K, Kochova P, Vrzalova J, Topolcan O, Treska V (2010). Stereological tools for quantitative microscopy of the aortic wall with focus on the abdominal aortic aneurysm. Microscopy: Science, Technology, applications and Education,; A. Mendez – vilas and Diaz J (Eds). Pp 926 – 929.
19. Villaschi S, Nicosia RF, Smith MR (1994). Isolation of a morphologically and functionally distinct smooth muscle cell type from the intimal aspect of the normal rat aorta: evidence for the smooth muscle cell heterogeneity *in vitro*. Cell and Developmental Biology 30:589-595.
20. Weyand CM, Krupa W, Pryscchep O, Groschel S, Bernadino R, Goronzy JJ (2005). Vascular Dendritic cell in Giant cell Arteritis. New York Academy of Sciences USA 1062: 195 – 208.
21. Wissler RW (1968). The arterial medial cell; smooth muscle or multifunctional mesenchyme. Journal of Atherosclerosis Research 8:201-213.
22. Zheng JW, Qiu WL, Zhang ZY; Lin GC; Zhu HG (2000). Anatomical and Histologic study of the cervical vessels in goats. Shangai Kou Qiang Yi Xue 9: 39 – 41.