Bulgarian Journal of Veterinary Medicine (2010), **13**, No 4, 211–217

SCLEROPROTEIN AND VASCULAR SMOOTH MUSCLE CELL DISTRIBUTION OF COMMON CAROTID MEDIA IN SHEEP AND GOATS

R. A. FATAHIAN DEHKORDI & A. PARCHAMI

Department of Anatomical Sciences, Faculty of Veterinary Medicine, University of Shahrekord, Shahrekord; Iran

Summary

Fatahian Dehkordi, R. A. & A. Parchami, 2010. Scleroprotein and vascular smooth muscle cell distribution of common carotid media in sheep and goats. *Bulg. J. Vet. Med.*, **13**, No 4, 211–217.

The aim of this study was to compare scleroprotein and smooth muscle cell distribution of common carotid media in sheep and goat. Ten adult sheep and ten adult goats were selected from the Shahrekord abattoir, Iran. Tissue sections showed that the media of the sheep and goat common carotid artery had similar stiffness but different elastic properties throughout its length, and different stiffness and elasticity throughout its thickness. The distribution of vascular smooth muscle cells was more uniform throughout the length and thickness of the common carotid media. The mean area fraction of both collagen and elastin and the mean number of smooth muscle cell nuclei per unit area (mm^2) of the different parts of the common carotid artery were numerically greater in goats in comparison with those in sheep but the differences were not statistically significant.

Key words: collagen, common carotid artery, elastin, goat, sheep

INTRODUCTION

Histological examination of blood vessels shows that in general, the elastic tissue is laid down in concentric layers, interspersed with collagen and circumferentially arranged smooth muscle cells. In the major arteries of mammals, the bulk of the vessel wall consists of the tunica media, which contains the elastic laminae. These are sandwiched between the tunica interna (a layer of endothelial cells and the underlying basal elastin layer) and the outer layer of predominantly collagen called the tunica adventitia (Shadwick, 1999). Arteries are composite structures that derive their non-linear properties from the combination of both rubbery and stiff fibrous constituents. The most studied arterial elastomer is elastin, which occurs in nearly all vertebrates (Sage & Gray, 1979). This rubber-like protein forms a highly extensible tissue that has an elastic modulus of approximately 1 MPa, comparable with that of an ordinary rubber band (Aaron & Gosline, 1980). Collagen is relatively inextensible and acts as a stiff reinforcing component. The elastic modulus of collagen fibres has not been measured directly, but tendons that are predominantly composed of parallel collagen fibres have elastic moduli greater than 1 GPa. Thus, collagen is more than 1000 times stiffer than elastin. The extracellular network formed by elastin and collagen is responsible for most of the mechanical strength of arteries (Torrance & Shwatz, 1961; Conklin *et al.*, 2002). The arterial media responds to alterations in mechanical stress by changing both its composition and morphology. These processes, whether they take place during normal growth and development or in response to pathological changes in pressure and flow, are mediated by the synthetic and contractile activity of the vascular smooth muscle cell (Stergiopulos *et al.*, 2001). It is well known that both experimentally induced and naturally occurring hypertension lead to increased collagen production and smooth muscle cell hypertrophy (Heath & Kay, 1967; Wiener *et al.*, 1977; Fung & Liu, 1989; Liu & Fung, 1989; Olivetti *et al.*, 1982; Baumbach & Ghoneim, 1993; Matsumoto & Hayashi, 1996).

The distributions of elastin and collagen fibres are known to be heterogenous through the arterial media. Demiray & Vito (1991) addressed the problem of elastic non-homogeneity by cutting the arterial wall circumferentially to produce two layers which were assumed to the media and adventitia. By measuring the elastic properties of each they found that the inner layer was stiffer than the outer and ascribed this discrepancy to differences in structure between them. Matsumoto *et al.* (1998) have shown that the stress distribution in the bovine thoracic aorta is not uniform although the strain distribution is which implies that the constitutive relation linking stress and strain must vary across the thickness of the wall. In many types of arteries, the concentration of collagen increases while that of elastin decreases from the intima to the adventitia. In bovine carotid arteries, the area fraction of collagen increased toward the outer layer in the media while that of elastin decreased in the same direction. (Hasan & Greenwald, 1995). In the aortas of children aged between 8 days and 12 years, medial elastin decreased while collagen increased from the intima to the adventitia (Feldman & Glagov, 1971). The similarity of the histological structures of the common carotid artery in goats and humans, makes it an appropriate animal model in experimental surgery (Zheng *et al.*, 2000). The aim of this study was to describe the distribution of collagen and elastic fibres and smooth muscle cells in the tunica media in different segments of common carotid artery in sheep and goat.

MATERIALS AND METHODS

Ten adult sheep and 10 adult goats of the Lori Bakhtiari breed were selected before their slaughter in the abattoir of Shahrekord. Immediately after slaughter, left sides of the cervical and thoracic regions were dissected and tissue samples were taken from the proximal, middle and distal parts of the left common carotid artery. A ring of approximately 2 mm long from each piece was fixed in 10% formal saline for 24 hours and embedded in paraffin wax using standard histological procedures. Three adjacent 5 µm sections were cut from the centre of each block. The first was stained with picrosirius red to reveal collagen, the second with Miller's elastic stain to visualize elastin and the third with Ehrlich's haematoxylin to stain cell nuclei for counting vascular smooth muscle cells.

The area fraction of collagen and elastin fibres of the inner and outer halves of the tunica media and the number of smooth muscle nuclei per unit area (mm^2) of the tunica media were measured using the methods previously described by Stergiopulos *et al.* (2001). The mean area fraction of collagen and elastic fibres and the mean number of smooth muscle cell nuclei per mm^2 between the two species were compared by Student's t-test and in different segments of the common carotid artery by Tukey's test. P<0.05 was considered as significant.

RESULTS

The area fraction of collagen and elastin fibres and the number of nuclei per mm^2 in the different segments of common carotid media in sheep and goat are presented on Fig. 1–3.

Fig. 1 shows that in both species the mean area fraction of collagen in the outer half of the proximal, middle and distal parts of the vessel (in sheep: 0.471±0.003, 0.478±0.004, 0.474±0.003; in goats: 0.481±0.015, 0.498±0.002, 0.482±0.002 respectively) were greater than those of the inner half (in sheep: 0.442±0.004, 0.438±0.003, 0.446±0.002; in goats: 0.446±0.002, 0.449±0.005, 0.454±0.004 respectively). The reverse was true about elastin (Fig. 2) – in both species the mean area fractions of elastin in the outer half of the proximal, middle and distal parts of the vessel (in sheep: 0.478±0.004, 0.448±0.004, 0.412±0.005; in goats: 0.482±0.003, 0.456±0.002, 0.423±0.003 respectively) were lesser than those of the inner half (0.491±0.002, 0.462±0.004, 0.428±0.003 in sheep; 0.496±0.002, 0.470±0.003, 0.432±0.003 in goats respectively).

Fig. 3 shows that in sheep and goats, the number of vascular smooth muscle cell nuclei per mm^2 of the proximal, middle and distal parts of the common carotid artery was numerically greater in the outer half of the media (in sheep: 1510±172, 1630±262. 1490±270; in goats: 1598±278, 1668±253, 1544±340 respectively) compared to the inner half (in sheep: 1480±190, 1583±248, 1460±213; in goats: 1570±200, 1610±273, 1490±199 respectively), but these differences were not statistically significant. In both species, the number of vascular smooth muscle cells in the different parts of the common carotid did not differ statistically.

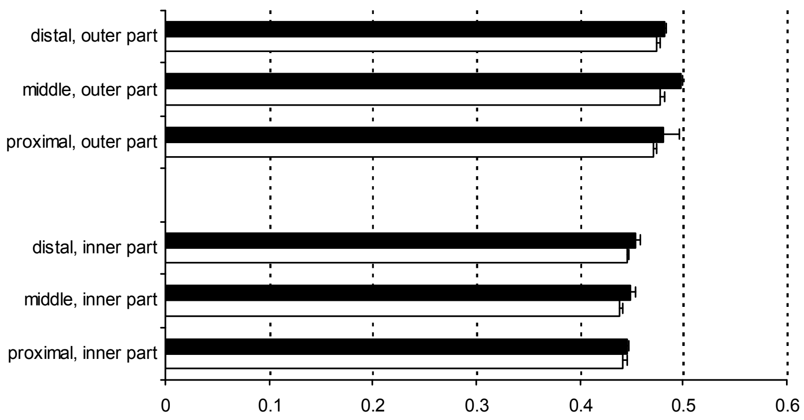

Fig. 1. Mean area fraction of collagen in the inner and outer halves of proximal, middle and distal parts of common carotid media in sheep (white bars) and goats (black bars). Data are presented as mean±SD.

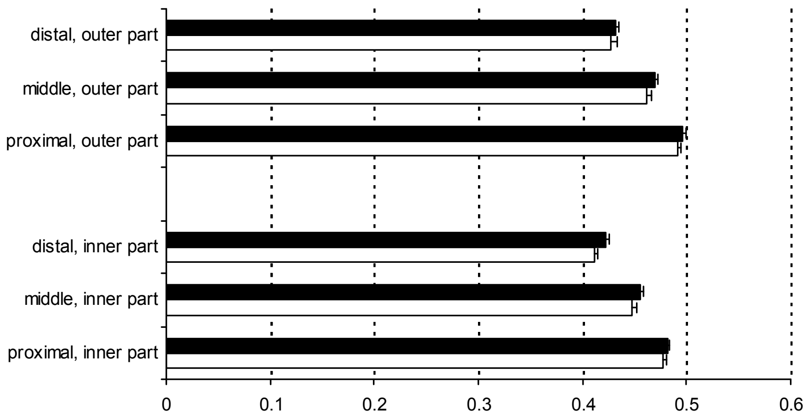

Fig. 2. Mean area fraction of elastin in the inner and outer halves of proximal, middle and distal parts of common carotid media in sheep (white bars) and goats (black bars). Data are presented as mean±SD.

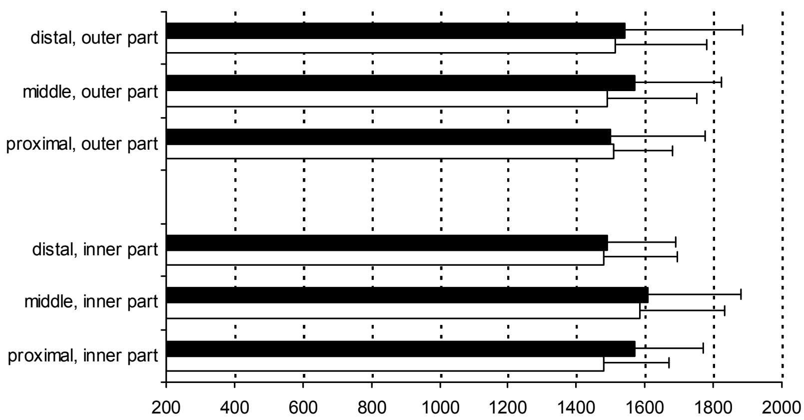

Fig. 3. Mean number of smooth muscle nuclei per mm^2 in the inner and outer halves of proximal, middle and distal parts of common carotid media in sheep (white bars) and goats (black bars). Data are presented as mean±SD.

Results obtained from this study also showed that the mean area fraction of both collagen and elastin and the mean number of smooth muscle cell nuclei per mm^2 of the common carotid media were numerically greater in goats in comparison with those in sheep but these differences were not statistically significant. There were no statistically significant differences between the area fraction of collagen in the proximal, middle and distal parts of the vessel. The mean area fraction of elastin decreased significantly from the proximal part of common carotid artery to its distal part in both species.

DISCUSSION

Results obtained from this study revealed that in both species the mean area fraction of collagen in the outer half of the proximal, middle and distal parts of the vessel were significantly greater than those of the inner half but the reverse was true about elastin. The extracellular matrix network formed by elastin and collagen fibres is responsible for most of the mechanical strength of arteries (Torrance & Shwatz, 1961; Conklin *et al.*, 2002). These distributions, measured optically, were not uniform across the arterial wall. Thus, the local mechanical property of an arterial wall was assumed to vary in proportion to the area fractions of elastin and collagen fibers (Kim, 2007). In many types of arteries, the concentration of collagen increases, while that of elastin decreases from the intima to the adventitia. In bovine carotid arteries, it was observed that the area fraction of collagen increased from 0.183±0.22 to 0.852±0.12 toward the outer layer in the media (Hasan & Greenwald, 1995). A similar pattern of an increasing medial collagen/elastin ratio from the intima toward the adventitia has been reported in the bovine aorta (Hasan & Greenwald, 1995). Feldman & Glagov (1971) have shown from biomechanical estimations on human aortas of young subjects that the collagen composition increased from the intimal side to the adventitial side while elastin decreased correspondingly. Kim (2007) stated that the area fraction of collagen fibres in the media was small and relatively uniform in the inner media and gradually increased toward the outer boundary of the media.

The area fraction of collagen fibres was the largest at the medial-adventitial border including the external elastic lamina. In the inner half of the arterial wall, collagen fibres were denser at the internal elastic lamina compared to the inner media (Feldman & Glagov, 1971; Merrilees *et al.*, 1987; Hasan & Greenwald, 1995; Stergiopulos *et al.*, 2001). The larger area fraction of collagen fibres in the outer media implies that structural stiffness is higher in the outer media than in the inner media i.e. the outer layer of the arterial media may provide more mechanical strength compared to the inner layer. Due to the cylindrical geometry and incompressibility, the increase of strain in response to the increase of pressure is always higher at the inner layer. Under physiologic conditions, the pulsatility of blood flow also causes higher strain fluctuation at the inner layer than the outer layer. Thus, higher structural stiffness in the outer layer compensates for low strain in the outer layer and makes the distribution of stress more uniform (Kim, 2007). In addition, the size of collagen fibrils progressively increases from the intima to the adventitia. Merrilees *et al.* (1987) reported mean diameters of collagen fibrils of 30–40 nm in the intima and inner media and 50–100 nm in the outer media of arteries from humans, pigs, and rats. Findings obtained from this study are inconsistent with those of Stergiopulos *et al.* (2001) who studied the homogeneity of the elastic properties and composition of the pig aortic media and stated that the mean area fraction of the elastin was greater in the outer half of tunica media than its inner half. It is clear, therefore, that there is a considerable variation in the degree of structural homogeneity between species and probably between differently aged subjects of the same species. Considering the differences in stiffness between elastin and collagen fibres, the intramural distribution of stress is more sensitive to the distribution of collagen fibres than elastin distribution. Results obtained from this study also revealed that there were no statistically significant differences between the area fraction of collagen in the proximal, middle and distal parts of the vessel which shows that the distribution of collagen content is more uniform across the length of the vessel wall in comparison with its thickness.

In both species the mean area fraction of elastin decreases significantly from the proximal part of the common carotid artery to its distal part. This is due to the fact that the artery distant from the heart gradually decreases its elastic lamellae in the tunica media as well as its elasticity (Awal *et al.*, 1999) and shows that different sites within the same specimen have different elastic properties and composition. In the light of non-availability of any report on the area fraction of collagen and elastin in the different parts of the same specimen, the present observations remain uncompared.

In sheep and goats, the mean number of vascular smooth muscle cell nuclei per mm^2 of the proximal, middle and distal parts of common carotid artery wass numerically greater in the outer half of the media than in its inner half, but these differences were not statistically significant. This observation is consistent with that of Stergiopulos *et al.* (2001) in pig aortic media who stated that there was no significant difference in the number of vascular smooth muscle cell per mm^2 when the inner and outer halves of the pig aortic media were compared. In both species the mean number of vascular smooth muscle cell nuclei per mm^2 of the tunica media in the different parts of the common carotid artery were not statistically different. These findings reveal that the distribution of vascular smooth muscle cells is more uniform throughout the length and thickness of the common carotid media.

ACKNOWLEDGMENT

This work was financially supported by the University of Shahrekord, Iran.

REFERENCES

Aaron, B. B. & J. M. Gosline, 1980. Optical properties of single elastin fibers indicate random protein conformation. *Nature*, **287**, 865–867.

Awal, M. A., M. A. A. Prodan, M. Asaduzzaman & M. Kurohmaru, 1999. Histological studies on the arterial walls of main arteries supplying the mammary glands of black Bengal goats (*Capra hircus*) in Bangladesh. *Veterinarski Arhiv*, **69**, 309–318.

Baumbach, G. L. & S. Ghoneim, 1993. Vascular remodeling in hypertension. *Scanning Microscopy*, **7**, 137–142.

Conklin, B. S., E. R. Richter, K. L. Kreutziger, D. S. Zhong & C. Chen, 2002. Development and evaluation of a novel decellularized vascular xenograft. *Medical Engineering & Physology*, **24**, 173–183.

Demiray, H. & R. P. Vito, 1991. A layered cylindrical shell model for an aorta. *International Journal of Engineering Science*, **29**, 47–54.

Feldman, S. A. & S. Glagov, 1971. Transmedial collagen and elastin gradients in human aortas: Reversal with age. *Atherosclerosis*, **13**, 385–394.

Fung, Y. C. & S. Q. Liu, 1989. Change of residual strains in arteries due to hypertrophy caused by aortic constriction. *Circulation Research*, **65**, 1340–1349.

Hasan, N. & S. E. Greenwald, 1995. Variation in the concentration of scleroproteins across the arterial wall. *The Journal of Pathology*, **176** (Suppl.), 26A.

Heath, D. & J. M. Kay, 1967. Medial thickness of pulmonary trunk in rats with cor pulmonale induced by ingestion of *Crotalaria spectabilis* seeds. *Cardiovascular Research*, **1**, 74–79.

Kim, Y. S., 2007. Correlation between MMP-

2 and -9 levels and local stresses in arteries using a heterogeneous mechanical model. A thesis presented to the Academic Faculty, School of Mechanical Engineering, Georgia Institute of Technology.

Liu, S. Q. & Y. C. Fung, 1989. Relationship between hypertension, hypertrophy and opening angle of zero-stress state of arteries following aortic constriction. *Journal of Biomechanical Engineering,* **111**, 325–335.

Matsumoto, T. & K. Hayashi, 1996. Stress and strain distribution in hypertensive and normotensive rat aorta considering residual strain. *Journal of Biomechanical Engineering*, **118**, 62–73.

Matsumoto, T., T. Sakamoto & M. Sato, 1998. Experimental analysis of the stress and strain distribution in the bovine thoracic aorta. In: *Proceedings of the Third World Congress of Biomechanics*, Sapporo, Hokkaido University, p. 46.

Merrilees, M. J., K. M. Tiang & L. Scott, 1987. Changes in collagen fibril diameters across artery walls including a correlation with glycosaminoglycan content. *Connective Tissue Research*, **16**, 237–257.

Olivetti, G., M. Melissari, G. Marchetti & P. Anversa, 1982. Quantitative structural changes of the rat thoracic aorta in early spontaneous hypertension. Tissue composition, hypertrophy and hyperplasia of smooth muscle cells. *Circulation Research*, **51**, 19–26.

Sage, E. H. & W. R. Gray, 1979. Studies on the evolution of elastin. I. Phylogenetic distribution. *Comparative Biochemistry and Physiology*, **64b**, 313–327.

Shadwick, R. E., 1999. Mechanical design in arteries. *The Journal of Experimental Biology*, **202**, 3305–3313.

Stergiopulos, N., S. Vulliémoz, A. Rachev, J. J. Meister & S. E. Greenwald, 2001. Assessing the homogeneity of the elastic properties and composition of the pig aortic media. *Journal of Vascular Research*, **38**, 237–246.

Torrance, H. B. & S. Shwatz, 1961. The elastic behaviour of the arterial wall. *Journal of the Royal College of Surgeons of Edinburgh*, **7**, 55.

Wiener, J., A. Loud, F. Giacomelli & P. Anversa, 1977. Morphometric analysis of hypertension-induced hypertrophy of rat thoracic aorta. *The American Journal of Pathology*, **88**, 619–634.

Zheng, J. W., W. L. Qiu, Z. Y. Zhang, G. C. Lin & H. G. Zhu, 2000. Anatomical and histologic study of the cervical vessels in goats. *Shanghai Kou Qiang Yi Xue*, **9**, 39–41.

Paper received 29.04.2010; accepted for publication 09.09.2010

Correspondence:

R. A. Fatahian Dehkordi
Department of Anatomical Sciences,
Faculty of Veterinary Medicine,
University of Shahrekord,
Shahrekord, Iran
tel/fax: +98 381 4424427
e-mail: fatahian_1349@yahoo.com