

COMPARATIVE HISTOMORPHOMETRIC STUDY OF THE COMMON CAROTID ARTERY AND ITS TERMINAL BRANCHES IN SHEEP AND GOATS

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Summary

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Light microscopic analysis of the mural structure of the common carotid artery (CCA), external carotid artery (ECA) and occipital artery (OA) in Lori Bakhtiari sheep and goats revealed variability in the average values of vascular diameter and the thickness of the intimal plus medial coats and adventitial coat. Based on the histological characteristics and organization of connective tissue fibres and smooth muscle cells in the tunica media, the arterial segments were clearly classified into elastic and muscular types. In both species the common and external carotid arteries were of the elastic type. The number of elastic lamellae in the tunica media of proximal, middle and distal parts of the CCA in both species was 11, 9 and 8 respectively; the number of these lamellae for ECA was 6. The artery distant from the heart gradually decreases its elastic lamellae in the tunica media as well as its elasticity. The OA was of the muscular type. In both species the largest mean value of vascular lumen diameter was determined for the CCA, followed by the ECA. The OA showed the smallest lumen diameter compared to both the CCA and ECA. The conjunct intimal plus medial layers, adventitial layer and lumen diameter were significantly greater in sheep in comparison to goats. The adventitial layer in all vessels were thicker in sheep than in goats but the differences were not statistically significant.

Key words: carotid artery, goat, morphometry, sheep

INTRODUCTION

The common carotid artery (CCA) usually divides at the retromandibular space at the cranial part of the neck, ventral to the wing of the first cervical vertebra (atlas), either into three branches, viz. external carotid artery (ECA), internal carotid artery (ICA) and occipital artery (OA) or into two branches where the internal carotid artery disappears in ruminants (Baldwin, 1964; Khamas & Mahdi, 1984; Khamas *et al.*, 1984). In sheep and goats, the CCA gives off the OA at the cranial

part of the neck between the caudal belly of the digastricus and stylohyoid muscles, making the transition between the CCA and ECA (Ghoshal, 1975).

Histomorphometric properties of the coronary arteries have been investigated in man (Dhall *et al.*, 2003; Ünlu *et al.*, 2003; Jashnani *et al.*, 2005), goats (Zheng *et al.*, 2000) and dogs (Orsi *et al.*, 2006). In men, the thickness of intima-media of CCA and the internal carotid artery were found to be good predictors of coronary

events. As carotid and coronary vascular beds share the same risk factors and manifest similar atherosclerotic changes, there is also a significant correlation between the carotid artery intima-media thickness and the percentage of block in the coronary arteries. Hence, considerable attention has been directed at the wall thickness of carotid arteries as an early marker of atherosclerotic disease and as a means of showing the effectiveness of medical therapies in the treatment of atherosclerosis (Jashnani *et al.*, 2005). Moreover, with regard to the histoarchitecture of some human elastic arteries including the CCA, some studies had demonstrated variability in the thickness of the adventitial layer as well as local differences in the thickness of the complex formed by the intimal plus medial layers, mainly in regions subjected to small but expressive haemodynamic stress (Willekes *et al.*, 1999). Histological studies of the arterial walls of miniature swine (Tanigawa *et al.*, 1985) and Wistar rats (Awal *et al.*, 1995) are also available. Similarity of the histologic structures of the common carotid artery in goats to that of humans makes it advantageous for use as an animal model in experimental surgery (Zheng *et al.*, 2000). The histomorphometric properties of the CCA and its terminal branches in the sheep and goats are not described in the available literature.

The present light microscopy study was therefore planned to compare the structural features of the above mentioned vessels in sheep and goats based on morphological and morphometrical analyses.

MATERIALS AND METHODS

Ten adult sheep and ten adult goats of the Lori Bakhtiari breed were selected before their slaughter in the abattoir of Shahre-

kord, Iran. After cervical and thoracic dissections, tissue samples were taken from the following sites: 1) Proximal, middle and distal parts of the left common carotid artery; 2) Left external carotid artery 1 cm from its origin and 3) Left occipital artery 1 cm from its origin.

The tissue samples were immediately fixed in 10% buffered formalin solution at room temperature for 24 hours. Next, the tissues were dehydrated in a series of ethanol of increasing concentrations and embedded in paraffin. Sections of 5 μ m thickness were obtained using microtome and stained by Verhoeff's elastin stain to distinguish collagen and elastic fibres (Humason, 1979). Three step-sections from each piece cut at 70 μ m interval were subjected to morphometric analysis using a microprojector (magnification $\times 40$). The profile of each section was drawn outlining carefully the endothelial lining, internal elastic lamina and outer margin of tunica media on a white sheet. Using appropriate grids for point-counting and linear-intercept techniques, the areas of tunica media plus tunica intima, tunica adventitia and lumen circumference were measured. Then, the thickness of the intimal plus medial layer, adventitial layer and lumen diameter in each vessel were calculated from these parameters as described by Aherne & Dunnill (1982). Elastic lamellae were counted (30 slides for each arterial segment) by using higher magnification ($\times 100$) and from the enlarged microphotographs as described by Awal *et al.* (1995). Classification of the arteries into belonging either to the elastic or to the muscular type was based on the histological characteristics and organization of connective tissue fibres and smooth muscle cells in the three distinct tunics: tunica intima, tunica media, and tunica adventitia as reported by Samuelson (2007). The thickness of each

layer and lumen diameters were compared between the two species using the Student's *t*-test, at level of significance 0.05.

RESULTS AND DISCUSSION

Table 1 compares the morphometric features of the common, external and occipital arteries in sheep and goats.

In both species the common carotid and external carotid arteries were of the elastic type (Fig. 1). The tunica intima consisted of a single layer of flattened endothelial cells resting on the internal elastic membrane. The subendothelial layer was predominantly composed of branched elastic and collagen fibres. These results revealed that the overall histomorphological features of the CCA and ECA as typical elastic arteries in goats and sheep were similar to that of the ascending aorta, aortic arch, brachiocephalic trunk and subclavian artery in goats (Awal *et al.*, 1999), the arterial segments from the ascending aorta to the thoracic aorta in miniature swine (Tanigawa *et al.*, 1985) and the arterial segments from the ascending aorta to the abdominal aorta in Wistar rats (Awal *et*

al. 1995). The tunica media of the elastic arteries was the thickest of the three tunicae and consisted of predominantly concentric layers of well-defined elastic lamellae. The number of elastic lamellae in the tunica media of proximal, middle and distal CCA parts in both species was 11, 9 and 8 respectively; and the number of these lamellae for ECA was 6. The existence of well-defined elastic lamellae in the wall of the arteries near to the heart is due to resistance of comparatively high arterial pressure compared to that of the muscular arteries (Awal *et al.*, 1999). The number of elastic lamellae in the tunica media depends on the size and relative distance of the arteries from the heart (Awal *et al.*, 1995) and also varies among domestic and laboratory animals (Awal *et al.*, 1997, 1998). The spaces between the elastic lamellae were occupied with connective tissue fibres and smooth muscle cells arranged circumferentially. The external elastic lamina was clearly defined. The tunica externa was comparatively thin and composed of connective tissue fibres, vasa vasorum, smaller blood vessels and nerves. Results obtained from this study revealed that the overall histomorphological properties of the tunica

Table 1. Values of various morphometric measurements of the common carotid artery (CCA), the external carotid artery (ECA) and the occipital artery (OA) in Lori Bakhtiari sheep and goats (Mean±SD; n=10)

Factors	Vessels	CCA			ECA	OA
		proximal	middle	distal		
Intima-media (µm)	sheep	430.00±38.68*	410.00±48.47*	450.00±49.49*	290.00±35.39*	170.00±20.00*
	goats	310.00±31.37	330.00±26.64	290.00±31.6	210.00±18.36	135.00±9.21
Adventitia (µm)	sheep	200.00±20.00	220.00±41.64	240.00±35.16	130.00±23.17	80.00±18.45
	goats	170.00±55.22	210.00±27.38	220.00±53.38	110.00±35.49	68.00±10.48
Lumen (mm)	sheep	2.51±0.22*	2.57±0.29*	2.74±0.34*	1.97±0.25*	0.97±0.05*
	goats	1.83±0.25	1.79±0.12	1.80±0.29	1.28±0.27	0.86±0.07

* statistically significant difference between both species (P<0.05).

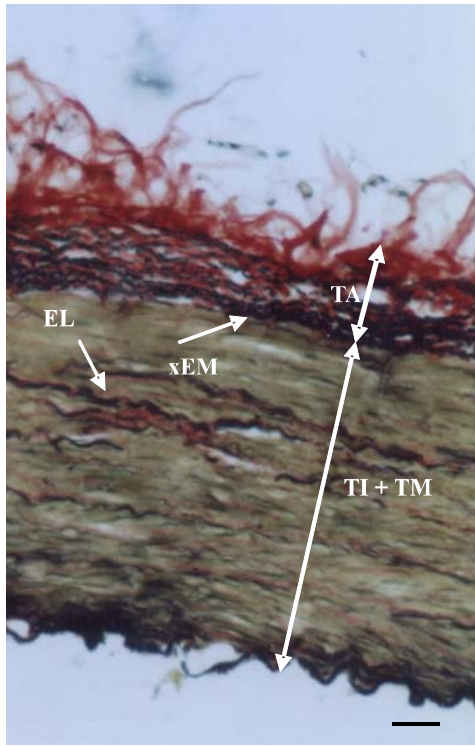


Fig. 1. External carotid artery. TA=tunica adventitia, TI=tunica intima, TM=tunica media, EL=elastic lamina, xEM=external elastic membrane; Verhoeff's elastin stain; bar=40 μ m.

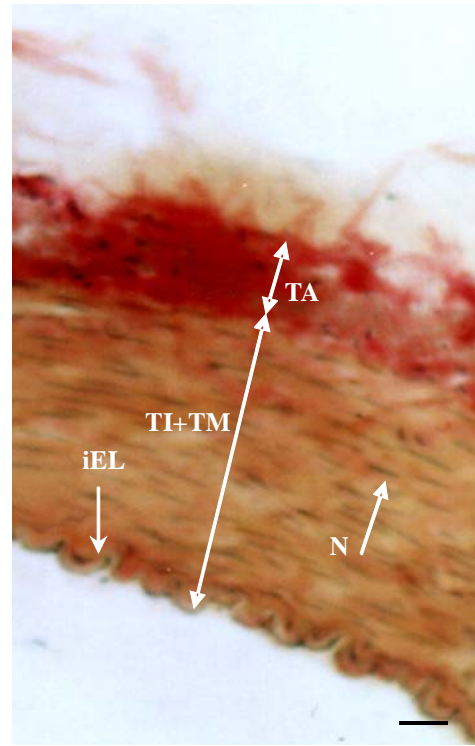


Fig. 2. Occipital artery. TA=tunica adventitia, TI=tunica intima; TM=tunica media; iEL= internal elastic membrane, N=smooth muscle cell nucleus; Verhoeff's elastin stain, bar=30 μ m.

externa of CCA and ECA as elastic arteries were similar to those found in arterial walls of main elastic arteries supplying the mammary glands of black Bengal goats that are typical elastic arteries (Awal *et al.*, 1999).

The occipital arteries in both species were of the muscular type (Fig. 2). The internal and external elastic laminae were present. The tunica intima consisted of a single layer of flattened endothelium resting on the internal elastic membrane. The tunica media, completely devoid of elastic lamellae, consisted mainly of smooth

muscle cells with a mixture of a few elastic fibres. The media was composed of circularly arranged smooth muscle cells with a small amount of fine elastic fibres. The internal elastic membrane was present and well developed, but the external elastic lamina was either indistinct or absent. Both internal and external elastic laminae were present in the muscular arteries of Wistar rats (Awal *et al.*, 1995). The tunica externa was well developed and consisted of predominantly collagen fibres with coarse elastic fibres and smooth muscle cells. Cross-sections of

small blood vessels and nerve bundle were also observed. The overall histomorphological properties of the occipital artery in this study were similar to those found in the deep femoral, pudendo-epigastric trunk, external pudendal, mammary, cranial, caudal and middle mammary, and the caudal superficial epigastric arteries in goats which are all classified as muscular type (Awal *et al.*, 1999). In the present study, the tunica media of the muscular arteries was the thickest of the three tunicae. The tunica media was comparatively thicker than the tunica externa (Table 1). In some animals like Wistar rats and miniature swine, the tunica media of some muscular arteries is thinner than the tunica externa (Tanigawa *et al.*, 1985; Awal *et al.*, 1995).

The morphometry of the intimal plus medial layers, adventitial layer and mean lumen diameters of ECA and OA and the three CCA segments in sheep and goats showed that the conjunct thickness of the intimal plus medial layers and lumen diameter were significantly thicker in sheep than in goats ($P < 0.05$). In both species the mean thickness of the intimal plus medial layers, adventitial layer and lumen diameter were greater in CCA than ECA and OA. The increased thickness of the intimal plus medial layer complex and luminal thickness observed in the CCA vs ECA and OA could be theoretically related to the mechanical adjustment of the arterial wall and to the local haemodynamic stress on the vascular wall layers, as in humans (Willekes *et al.*, 1999.) and canine CCA (Orsi *et al.*, 2006). The adventitial layer in all vessels was numerically thicker in sheep than in goats but the differences were not statistically significant ($P > 0.05$). The fact that there was no statistically significant difference in the adventitial thickness between the two species indicates that the effect of the

animal species on the mechanical adjustment of the arterial wall has probably greater influence on the tunica media thickness than that on the adventitia. Concerning the lumen diameter of the arteries of both species, the largest mean vascular lumen diameter was observed for the CCA, followed by the ECA with an intermediate diameter value. The OA showed the smallest lumen diameter as compared to CCA and ECA.

In conclusion, the results of the present study showed that in sheep and goats, the CCA and ECA were of the elastic type and the OA of the muscular type. The elastic arteries away from the heart gradually lose their elastic lamellae in the tunica media. In both species the largest mean value of vascular lumen diameter was determined for the CCA, and the smallest value for the OA. The conjunct intimal plus medial layers, adventitial layer and lumen diameter were greater in sheep in comparison to goats. The adventitial layer in all vessels were thicker in sheep than in goats.

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