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Quantitative CT imaging of the spatio-temporal distribution patterns of vasa vasorum in aortas of apoE$^{-/-}$/LDL$^{-/-}$ double knockout mice

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ABSTRACT

Objective: To investigate the distribution of vasa vasorum (VV) relative to advanced atherosclerotic lesions (calcified, fibrotic or hemorrhaged) along the aortic wall of apoE$^{-/-}$/LDL$^{-/-}$ mice at the age of 25 and 80 weeks using high-resolution nano-CT.
Methods: Aortas from male apoE$^{-/-}$/LDL$^{-/-}$ mice at the age of 25 weeks (n = 4) and 80 weeks (n = 7) were infused in situ with contrast agent and harvested for scanning with nano-CT. The spatial distribution of vasa vasorum [number and area/cross-section (mm^2)] was compared to aortic luminal cross-sectional area and plaque cross-sectional area in the ascending aorta, aortic arch and descending aorta. Results were complemented with co-localized histology.
Results: The number and total luminal cross-sectional area of VV showed a significant decrease in the ascending aorta and aortic arch from 25 to 80 weeks but not in the descending aorta. The number and cross-sectional area of VV showed significant local differences depending on whether it was near a fibrotic, and hemorrhaged or calcified plaque in animals at the age of 80 weeks. Area of VV progressively increased along the aorta from least in the ascending aorta < aortic arch < descending aorta in animals at the age of 80 weeks and is inverse in animals aged 25 weeks.
Conclusion: Atherosclerotic lesion type is correlated to the number and cross-sectional area of VV in different aortic segments in apoE$^{-/-}$/LDL$^{-/-}$ mice. The chronological development of VV along the aorta proceeds distally from the ascending aorta and aortic arch.

1. Introduction

Substantial variations in the development of atherosclerotic lesions have been described in the arterial vasculature [1–3]. Although a large variety of different effects (i.e. pulse pressure, shear stress, vessel wall diameter) may contribute to lesion formation in different vascular beds [4–6] the underlying mechanism(s) of this heterogeneous response is not yet fully understood.

The association between adventitial VV and in atherogenesis has been demonstrated [7–9]. Moreover, it has been shown that the spatial location and magnitude of VV differs in different vascular beds in apoE$^{-/-}$/LDL$^{-/-}$ double knockout mice [10]. In that study the spatial location and magnitude of vasa vasorum (VV) density and adventitial inflammation were shown to be strongly correlated in advanced atherosclerotic lesions and identified as independent correlates to different categories of advanced lesion types.

However, none of these studies addressed the chronological development and distribution pattern in the same artery.

The present study was designed to investigate the number and luminal cross-sectional area of vasa vasorum and their relation to atherosclerotic plaques along the total thoracal aorta of apoE$^{-/-}$/LDL$^{-/-}$ double knockout mice at the age of 25 and 80 weeks. To localize, identify and quantitate the distribution of VV and atherosclerotic lesion formation in the entire thoracal aorta, we used nano-CT imaging as well as histology. Atherosclerosis in this animal model closely resembles human atherosclerosis and therefore represents an interesting animal model to investigate the chronological development of the association between vasa vasorum and atherosclerosis.

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2. Methods

2.1. Experimental design

Animal studies were performed according to the "German Animal-Protection Law" (1993). Approval of the institutional animal care and use committee was obtained before the start of this study.

Male apoE$^{-/-}$/LDL$^{-/-}$ double knockout mice (Charles Rivers Wiga, Sulzbach, Germany) on a Western-diet were euthanized after 25 weeks ($n=4$) and 80 weeks ($n=7$) with a fatal dose of inhaled trichlormethane. The left ventricle was cannulated and infused with heparinized saline (10 ml of 0.9% sodium chloride with 1000 IU Heparin) until the venous effluent was free of blood. A lead-containing radiopaque polymer (Microfil® MV-122, Flow Tech, Carver, MA, USA) was infused into the aorta at a nominal pressure of 100 mm Hg. After polymerization of the compound, the entire aorta was removed and immersed in 4% neutral buffered formalin.

2.2. Micro- and Nano-CT

First, the entire heart and thoracic aorta were scanned en-bloc using a micro-CT system (Micro-CT_1072) manufactured and developed by SkyScan® (Kontich, Belgium) at 14 μm voxel resolution with an 8-bit gray-scale range.

Next, the aorta was cut into segments (~10 mm length, ascending aorta, aortic arch and descending aorta), each of which was scanned using a nano-computed tomograph (Nano-CT_2011), manufactured and developed by SkyScan® (Kontich, Belgium). The microfocus X-ray source is a pumped type source (open type X-ray source) with a lanthanum boride cathode. The electron beam is focused by two electromagnetic lenses onto the surface of an X-ray target. The X-ray target (Au) contains a thin tungsten film plated on the surface of the beryllium window producing X-ray emission with a minimum spot size of <400 nm. The X-ray detector consists of a 12-bit digital, water-cooled CCD high-resolution (1280 × 1024 pixel) camera with fibre optic 3.7:1 coupling to an X-ray scintillator and digital frame-grabber. In our experimental setting samples were positioned on a computer controlled rotation stage and scanned 180° around the vertical axis in rotation steps of 0.5° at 40 kVp. Acquisition time for each view was 2.4 s. Multiple projection image data were reconstructed with a modified Feldkamp cone-beam reconstruction modus resulting in two dimensional 8-bit gray-scale images consisting of isotropic cubic voxels. Samples were scanned with a cubic voxel resolution of 1.2 μm or 390 nm on a side. This system has been described recently [11]. Measurements were performed using the ANALYZE©software package (ANALYZE 9.0, Mayo Clinic, Rochester, MN, USA). The number of VV was quantified in aortal cross-sections with fibrotic plaques ($n=274$, $n=182$; 25 and 80 weeks, respectively), calcified plaques ($n=28$, $n=172$; 25 and 80 weeks, respectively) and plaques with intraplaque hemorrhage ($n=345$; 80 weeks). Aortic lumen diameter (mm; 15 aortal segments, $n=401$ cross-sections; 11 aortal segments; $n=420$ cross-sections; 25 and 80 weeks, respectively), luminal- and plaque area (mm^2; 15 aortal segments, $n=401$ cross-sections; 11 aortal segments, $n=420$ cross-sections; 25 and 80 weeks, respectively) were measured in the ascending aorta, aortic arch and descending aorta.

2.3. Histology

Sections through areas with calcified lesions detected previously in the specimen's micro-CT images were stained with 3% silver nitrate (von Kossa's reagent) followed by a 0.1% nuclear fast red counterstaining. Contiguous serial sections (6 μm slice thickness) within each sample were prepared to detect iron by staining with Perls' Prussian blue reaction with 3,3′-diaminobenzidine

Table 1
Vasa vasorum distribution in different plaque types, aortic segments and age.

	# VV/cross-section	VV cross-sectional area (mm²)	Vessel Diameter (mm)	Plaque cross-sectional area (mm²)	Aortic Luminal cross-sectional area (mm²)
25 Weeks					
Fibrotic Plaque	3.9 ± 2.4	0.01 ± 0.009	1.04 ± 0.19	0.48 ± 0.28	0.39 ± 0.11
	n.s.	n.s.	p < 0.0001	p < 0.0001	p < 0.0001
Calcified Plaque	3.14 ± 2.05	0.009 ± 0.006	1.47 ± 0.07	1.07 ± 0.2	0.62 ± 0.07
Fibrotic Plaque with Intraplaque Hemorrhage	-	-	-	-	-
Ascending Aorta	4.38 ± 2.71	0.01 ± 0.0019	1.09 ± 0.29	0.53 ± 0.41	0.48 ± 0.16
	p = 0.01	p = 0.001	p < 0.0001	p < 0.0001	p < 0.0001
Aortic Arch	3.77 ± 2.48	0.01 ± 0.01	1.27 ± 0.16	0.84 ± 0.24	0.44 ± 0.11
	n.s.	p = 0.003	p < 0.0001	p < 0.0001	p < 0.0001 p<0.02
Descending Aorta	3.63 ±2.14	0.007 ± 0.006	0.93 ± 0.09	0.32 ± 0.14	0.36 ± 0.07
80 Weeks					
Fibrotic Plaque	6.8 ± 1.9	0.02 ± 0.02	1.22 ± 0.12	0.85 ± 0.15	0.31 ± 0.08
	n.s.	n.s.	p < 0.0001	p < 0.0001	n.s.
Calcified Plaque	1.4 ± 1.3	0.003 ± 0.005	1.67 ± 0.31	1.15 ± 0.37	1.10 ± 0.56
	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001
Fibrotic Plaque with Intraplaque Hemorrhage	6.9 ± 2.4	0.02 ± 0.01	1.33 ± 0.12	0.99 ± 0.21	0.35 ± 0.07
Ascending Aorta	2.6 ± 1.1	0.003 ± 0.002	1.97 ± 0.09	1.32 ± 0.17	1.73 ± 0.3
	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001
Aortic Arch	1.3 ± 1.3	0.01 ± 0.01	1.72 ± 0.12	1.42 ± 0.25	0.93 ± 0.32
	p < 0.0001 p = 0.03	n.s.	p < 0.0001	p < 0.0001 p = 0.03	p < 0.0001
Descending Aorta	6.0 ± 3.0	0.02 ± 0.002	1.29 ± 0.12	0.90 ± 0.21	0.57 ± 0.48