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Original Article

Adverse remodeling of the obtuse marginal artery in compensatory hypertrophied myocardium from spontaneously hypertensive rats

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ABSTRACT

Background: Spontaneously hypertensive rats (SHR) serve as a model of genetic hypertension. Adverse remodeling of a coronary artery has been reported in SHR. This model is used to study new therapies in regression vascular remodeling. However, no data are available that show remodeling of the intramyocardial branch of the obtuse marginal artery in 10-month-old SHR. This study was designed to assess remodeling (changes in vascular structure and fibrosis) of this coronary artery.
Methods and results: The study was performed on 10-month-old male SHR (n=7) and normotensive control Wistar Kyoto rats (WKY) (n=7). Using histology, we show that the external diameter, lumen diameter, wall width, and cross-sectional area of the intramyocardial artery were significantly greater in SHR than in WKY. The wall-to-lumen ratio was similar in SHR and WKY. The collagen volume density of the intramyocardial artery in SHR was significantly greater than in WKY.
Conclusions: Our results show hypertrophic outward remodeling in the intramyocardial branch of the obtuse marginal artery of the left ventricle in SHR. This artery can serve as a new vascular bed from adult SHR to study novel therapies in regression coronary artery remodeling.

1. Introduction

Hypertension-induced left ventricular hypertrophy (LVH) is an independent risk factor for myocardial ischemia, dysfunction, and heart failure. Structural remodeling of small arteries is one of the most prevalent forms of target organ damage in hypertension [1]. Intramyocardial artery remodeling (structural alteration) is responsible for impaired coronary reserve and increases the incidence of myocardial infarction and dysfunction. Spontaneously hypertensive rats (SHR) represent the animal model used most often for human essential hypertension [2]. Adult SHR present compensated LVH, which is associated with structural alterations of the coronary artery [3]. Previous studies have shown vascular remodeling in SHR in different vascular beds (aorta, left descending coronary artery, small arteries subcutaneous, mesenteric, carotid, femoral, and cerebral vessels). However, structural alterations of the obtuse marginal artery (intramyocardial branch) in adult SHR have not yet been investigated.

In the present study, we analyze the intramyocardial branch of obtuse marginal artery remodeling (vascular structure and fibrosis) in 10-month-old SHR.

2. Methods

All procedures fulfilled the stipulations of the Guide for the Care and Use of Laboratory Animals (Directive 2010/63/UE and RD 53/2013) and were approved by the Ethics Committee of Hospital General Universitario Gregorio Marañon, Madrid, Spain. The animals studied were 10-month-old male SHR (n=7) and normotensive Wistar Kyoto rats (WKY, n=7). They were bred at the animal research facility of Universidad Autónoma (Madrid, Spain) (EX021-U). Their systolic blood pressure (SBP) and heart rate (HR) were measured using the tail-cuff method with a photoelectric sensor (Niprem 546; Cibertec, Madrid, Spain). The rats were then euthanized by cervical dislocation. The hearts were immediately removed in order to study intramyocardial artery remodeling. Left ventricular tissue was then fixed in 4% sodium-buffered formaldehyde. We made sequential transverse cuts of the left ventricle from the subvalvular region to the apex.

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Table 1
Structural parameters of the intramyocardial branch of obtuse marginal artery in WKY and SHR

	WKY (n=7)	SHR (n=7)	P value
ED (μm)	180±14	360±21***	<.001
LD (μm)	93±16	196±28**	.009
WW (μm)	43±2	81±7**	.002
W/L (%)	55±8	49±11	.719
CSA (μm^2)	18599±2061	70373±6911***	<.001
CD (%)	14±1	46±2***	<.001

Data are expressed as mean ± S.E.M. Statistically significant differences between WKY and SHR are shown (**P<.01 vs. WKY; ***P<.001 vs. WKY). CD, collagen density.

Samples were then dehydrated and embedded in paraffin. Serial sections (5 μm) were stained with orcein. We differentiated between the subepicardial and midmyocardial parts of the left ventricular wall following the procedure described by Lunkenheimer et al. [4]. We then located the intramyocardial branch of the obtuse marginal artery (branch of the circumflex coronary artery) of the left ventricle. The intramyocardial artery was analyzed using a high-resolution camera (Sony CCD IRIS) attached to a microscope (Leica DMLB, 40× objective). The morphometric analyses were performed using the method of Gundersen et al. [5]. The images were projected on a computer screen, and the external diameter (ED) (lumen diameter+tunica intima+tunica media+tunica adventitia) and lumen diameter (LD) of the coronary arteries were measured. The wall width (WW) was expressed as (ED−LD)/2. The wall-to-lumen ratio (W/L) was expressed as (WW/LD)×100, and the media cross-sectional area (CSA) (tunica intima+tunica media+tunica adventitia) was expressed as $(\pi/4)\times(ED^2-LD^2)$ [6]. For collagen staining of the intramyocardial arteries, 5-μm sections of paraffin blocks were stained with Picrosirius red, and for each image, collagen volume density was determined (20× objective) using the method of Gundersen et al. [5].

The results were expressed as the mean±S.E.M. The parameters were compared using the t test for independent samples. P values <.05 were considered statistically significant. The statistical analysis was performed using IBM SPSS Statistics software for Windows, version 20.0 (IBM Corp., Armonk, NY, USA) and S-PLUS 6.1.

3. Results

3.1. Physiological parameters

Body weight was significantly higher in WKY than in SHR (407.12±9.25 g vs. 371.25±16.12 g, P<.05). SBP was significantly higher in SHR than in WKY (221±14 mmHg vs. 135±21 mmHg, P<.001). HR remained unchanged in WKY and SHR (291±15 beats/min vs. 285±13 beats/min).

3.2. Histological parameters

For intramyocardial artery structure (Table 1, Fig. 1), ED was significantly greater in SHR than in WKY (360±21 μm vs. 180±14 μm, P<.001), LD was significantly greater in SHR than in WKY (196±28 μm vs. 93±16 μm, P<.01), WW was significantly higher in SHR than in WKY (81±7 μm vs. 43±2 μm, P<.01), W/L ratio in SHR did not differ from that shown in the WKY (49±11% vs. 55±8%), and CSA was larger in SHR than in WKY (70373±6911 μm^2 vs. 18599±2061 μm^2, P<.001). Collagen density of the intramyocardial artery was significantly greater in SHR than in WKY (46±2% vs. 14±1%, P<.001) (Table 1, Fig. 2).

4. Discussion

4.1. SHR and coronary artery remodeling

Our results show structural alterations (geometry and fibrosis) in the intramyocardial branch of the obtuse marginal artery (branch of the circumflex coronary artery) in 10-month-old SHR. Previous studies have shown vascular remodeling in SHR but in other vascular beds (aorta, left descending coronary artery, small arteries subcutaneous, mesenteric, carotid, femoral, and cerebral vessels). Structural changes in other coronary arteries (septal branch of the left descending coronary artery) vary in SHR depending on age (3-week-old SHR showed no changes in WW, LD, or CSA; 52-week-old SHR showed changes in WW, LD, and CSA) [3]; however, we could not define the structural changes in 10-week-old SHR for this artery. Our results (WW, CSA, and LD were higher in SHR than WKY) are comparable with the results of Cebova et al. [3] concerning the septal branch of the left descending coronary artery of 52-week-old SHR.

Vascular remodeling in the presence of hypertension implies not only structural changes in the coronary artery but also endothelial dysfunction [7]. Structural and endothelial disease is variable in SHR owing to differences in age, sex, artery type, and methodology used for studying the remodeling of the coronary artery [8].

Coronary artery remodeling is responsible for impaired coronary reserve and increases incidence of myocardial infarction. Each coronary artery irrigates different areas of the heart. The septal branch of the left descending coronary artery irrigates the interventricular septum; however, the obtuse marginal artery (branch of the circumflex coronary artery) supplies the lateral and posterior area of the left ventricle. This is important in humans because when myocardial ischemia occurs, an electrocardiogram can quickly indicate the segment affected by the heart infarction, making it possible to identify the coronary artery that has caused myocardial ischemia. The exact location of the myocardial ischemia (and therefore, the damaged coronary artery) determines the prognosis of patients.

The gradual increase in all three parameters (WW, CSA, and LD) with age in coronary artery hypertrophy in SHR may reflect an adaptive mechanism triggered by chronic arterial hypertension. On the other

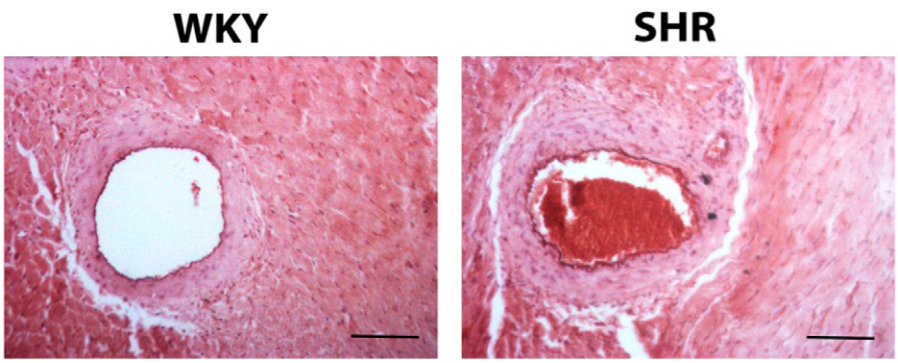

Fig. 1. Examples of histological sections (vascular structure) of the intramyocardial branch of obtuse marginal artery of the left ventricle from WKY and SHR. Orcein 100× (10 μm).