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Myocardial capillary supply is limited in hypertrophic cardiomyopathy: A morphological analysis ☆

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Abstract

Objective: To clarify the morphological basis of the limited coronary reserve in hypertrophic cardiomyopathy (HCM).
Background: Some of the symptoms in Hypertrophic cardiomyopathy (HCM), such as chest pain, dyspnea and arrhythmia, may be explained by myocardial ischemia. Many patients with HCM are known to exhibit these symptoms in the absence of atherosclerosis in the major coronary vessels. Decreased myocardial perfusion has been demonstrated in HCM, however, little is known about the myocardial capillary morphology in this disease.
Methods: Using immunohistochemistry and morphometry, we analysed capillaries and cardiomyocytes in myectomy specimens from 5 patients with HCM with moderate hypertrophy and left ventricular outflow tract obstruction and in 5 control hearts.
Results: The number of capillaries per cardiomyocyte ($p<0.009$) and number of capillaries per cardiomyocyte area unit, reflecting cardiomyocyte mass ($p=0.009$), were lower in individuals with HCM, *i.e.* indicating loss of capillaries. In HCM, the capillary density was 33% lower ($p<0.05$).
Conclusions: Our morphologic findings show that the capillary supply, and thus the coronary reserve, is impaired in HCM with moderate hypertrophy and left ventricular outflow tract obstruction. These data may partly explain the limitation of myocardial perfusion in HCM, which is associated with worse prognosis. Furthermore, we present evidence of actual loss of myocardial capillaries in HCM and a defective capillary growth.

Keywords: Cardiomyopathy; Hypertrophy; Capillaries; Myocytes; Immunohistochemistry

1. Introduction

According to World Health Organization criteria, hypertrophic cardiomyopathy (HCM) is characterised by left and/or right ventricular hypertrophy, with predominant involvement of the interventricular septum [1]. HCM is genotypically and phenotypically heterogeneous, with a variety of clinical manifestations, ranging from asymptomatic individuals to severe symptoms and early death [2]. Furthermore, HCM is an important cause of sudden cardiac death in children and young adults.

Hypertrophy of the upper interventricular septum may cause a fixed or dynamic obstruction of the left ventricular outflow tract, sometimes associated with systolic anterior motion of the anterior mitral leaflet and mitral regurgitation. Symptoms of left ventricular outflow obstruction can be reduced or even relieved by atrioventricular pacing [3], surgical septal myectomy [4] or alcohol septal ablation [5]. Patients with HCM, however, may also exhibit symptoms consistent with ischemia in the absence of coronary atherosclerosis [6]. Recent functional studies on myocardial perfusion using positron-emission tomography demonstrate

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reduced coronary flow reserve, a feature that also is associated with poor outcome [7]. There are some reports on narrowing of arterioles in HCM [8,9] whereas only little is known about myocardial capillaries [8]. In the present investigation, we focus on the intramyocardial capillaries and their distribution in relation to cardiomyocytes and cardiomyocyte size.

2. Materials and methods

2.1. Patients and tissues

Five individuals with hypertrophic cardiomyopathy and significant left ventricular outflow tract obstruction were included in the study, three women and two men, aged 28 to 73 yr. One of them, subject 4, had known family history of HCM with a mutation in the cardiac myosin binding protein C gene, a mutation known to cause HCM [10]. The four other cases had no known family history of HCM. No genetic analysis was performed in these four cases. All subjects reported symptoms consistent with the diagnosis of HCM and exhibited significant left ventricular outflow tract obstruction, necessitating myectomy. All patients underwent physical examination, echocardiography (M-mode, two-dimensional and Doppler) and 12-lead electrocardiogram. Echocardiographic evaluation was performed with an Acuson Sequoia or Acuson Cypress cardiac ultrasound system (Acuson, Mountain View, CA, USA). All measurements were done according to the guidelines of the American Society of Echocardiography [11]. Criterion for the diagnosis of HCM was left ventricular hypertrophy, with a diastolic wall thickness of ≥ 15 mm [1]. The demographic data and clinical characteristics of the patients are presented in Table 1. Informed consent was obtained from each patient. Samples from left ventricular myocardium were obtained after surgical myectomy. Control myocardium from the left ventricle was collected at autopsy of previously healthy individuals that suffered a sudden unnatural death. The protocol was approved by the Ethics Committee of the Medical Faculty, Umeå University. The autopsy specimens were collected in agreement with Swedish laws and regulations on autopsy and transplantation. All myocardial specimens were mounted in OTC compound (Tissue Tek®, Sakura Finetek, Zoeterwoude, Netherlands) and frozen in liquid propane chilled with liquid nitrogen. Until further processing, the tissues were stored at −80 °C.

2.2. Immunohistochemistry

Serial 5 μm thick sections were cut in a cryostat microtome (Leica CM 3050, Leica instruments GmbH, Nussloch, Germany) at −20 °C and mounted on glass slides. Identification of the cardiomyocyte and capillary cell borders (*i.e.* basement membranes) was performed using immunohistochemical staining with antibodies against the laminin α5-chain (mAb 4C7) [12,13] according to the peroxidase–antiperoxidase (PAP) technique. The mAb 4C7 labels the basement membrane of muscle cells and capillaries as previously described [14]. For routine examination, all sections were stained with Hematoxylin-eosin.

2.3. Morphometric analysis

All measurements were performed by a single investigator blinded for the origin of the samples. The intraobserver reproducibility using this analysis system and method is high and in our laboratory, the method error was only 2% in duplicate measurements at 24 h intervals [15]. The sections were positioned in the coordinate system of a light microscope stage (Zeiss Axiophot, Carl Zeiss, Oberkochen, Germany). Via a CCD camera, the microscope was connected to an image analysis system (IBAS, Kontron elektronik GMBH, Eching, Germany). For capillary analysis, 6 randomly chosen areas were scanned in each specimen. The morphometric data was based on the evaluation of 1455 cardiomyocytes and 1152 capillaries in the 5 hypertrophic cardiomyopathy patients and 2042 cardiomyocytes and 2375 capillaries in the 5 controls. To estimate the cardiomyocyte

Table 1
Clinical characteristics of patients with hypertrophic cardiomyopathy

Subject	Gender	Age (yr)	Age at diagnose (yr)	Blood pressure (mm Hg)	Symptoms	ECG	Echocardiography							
							LA (mm)	IVSD (mm)	LVPWD (mm)	IVSD/ LVPWD	LVEDD (mm)	LVESD (mm)	FS (%)	LVOT -obstruction
1	F	45	43	140/90*	Dyspnea	LVH	36	20	20	1.0	38	12	68	60–70 mm Hg at rest 160 mm Hg at stress
2	F	28	27	120/80	Dyspnea Fatigue	LVH	49	21	14	1.5	43	18	58	134 mm Hg at rest
3	F	73	66	130/85*	Dyspnea	ST-depression	43	19	12	1.6	40	21	48	16 mm Hg at rest 200 mm Hg at stress
4	M	43	41	120/70	Dyspnea	LVH	33	19	10	1.9	39	24	38	100 mm Hg at rest
5	M	68	66	140/80	Dyspnea	LBBB	52	20	11	1.8	46	30	35	50 mm Hg at rest

LA= Left atrium, IVSD= interventricular septum diameter in enddiastole, LVPWD = left ventricular posterior wall diameter in enddiastole, LVEDD = left ventricular enddiastolic diameter, LVESD = left ventricular endsystolic diameter, FS = fractional shortening, LVOT = left ventricular outflow tract, * indicates individuals treated for essential arterial hypertension (The blood pressure data is based on lowest and representative measurements in medical records), LVH = left ventricular hypertrophy, defined as Romhilt–Estes score ≥ 4 points, LBBB = left bundle branch block.