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Original Article

Circulating mesenchymal stem cells in patients with hypertrophic cardiomyopathy

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ABSTRACT

Background: This study examines the mobilization of mesenchymal stem cells (MSCs) in patients with hypertrophic cardiomyopathy (HCM) compared to healthy individuals. The pathogenesis of myocardial hypertrophy in HCM is not fully understood. MSCs are involved in the process of neovascularization, fibrosis, and ventricular wall remodeling.

Methods and results: We included 40 patients with HCM and 23 healthy individuals. Using flow cytometry, we measured MSCs in peripheral blood, as a population of CD45−/CD34−/CD90+ cells and also as a population of CD45−/CD34−/CD105+ cells. The resulting MSC counts were expressed as percentages of the total cells. Patients with HCM were found to have a greater percentage of circulating CD45−/CD34−CD34−/CD90+ cells compared to controls (0.0041±0.005% vs. 0.0007±0.001%, respectively, $P<.001$). No significant difference in circulating CD45−/CD34−/CD105+ cells in the peripheral blood was found between HCM patients and controls (0.016±0.018% vs. 0.012±0.014%, respectively, $P=.4$). Notably, circulating CD45−/CD34−/CD90+ cells were positively correlated with left ventricular mass index ($r=0.54$, $P<.001$).

Conclusions: Patients with HCM reveal an increased mobilization of MSCs compared to healthy individuals. Although further research is needed to reveal the clinical significance of our findings, our data open a new dimension in the pathophysiology of the disease and may indicate new future therapeutic possibilities.

1. Introduction

Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiomyopathy, with a prevalence of 0.2% [1]. HCM has a complex pathophysiology and a variable clinical outcome. It is characterized by the presence of unexplained left ventricular (LV) hypertrophy in the absence of another cause — for example, arterial hypertension that could lead to secondary hypertrophy. The predominant pathological features of HCM are myocyte hypertrophy and disarray as well as interstitial fibrosis [2].

HCM includes a wide spectrum of disease processes with complex pathophysiology and various clinical presentations and prognoses. Although our knowledge of this disease has progressed, it is still not completely understood. On the other hand, HCM is characterized by an impressive phenotypic heterogeneity, which ranges from negligible to extreme hypertrophy with or without LV outflow tract obstruction, even in patients carrying the same pathogenic HCM mutation [3]. Therefore, we need to acquire a better understanding of the mechanisms that are associated with the clinical manifestations of HCM, in order to improve the therapeutic management and the prognosis in this group of patients.

Mesenchymal stem cells (MSCs) are a group of heterogeneous multipotent cells that have sparked the interest of researchers because of their ability to self-renew and differentiate into many different cell types. MSCs have a highly plastic differentiation potential that includes not only adipogenesis, osteogenesis, and chondrogenesis but also endothelial, cardiovascular [4], and neovascular differentiation [5–7]. Although present in only very small numbers in peripheral blood, they are thought to be of great clinical significance in the pathophysiology of heart failure and atheromatosis. Previous studies have indicated that MSCs derived from peripheral blood, apart from their multilineage potential, can also be used for cellular and gene therapies [8]. In many pathological situations, there are reports in the literature of increased mobilization and recruitment of progenitor stem cells under various pathological cardiovascular conditions [9]. We already know that

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extracardiac stem cells of bone marrow origin are involved in the pathophysiology of cardiac hypertrophy in response to myocardial environmental cues [10]. For example, an increase in preload leads to the mobilization of progenitor cells from bone marrow for use in neovascularization in the heart, which plays an important role in cardiac hypertrophy [11]. On the other hand, the recruitment of bone-marrow-derived cells is involved in LV hypertrophy in response to pressure overload [12]. Given these findings, bone-marrow-derived progenitor stem cells might also be expected to play a part in the pathophysiology of the hypertrophy seen in HCM.

The aim of this study was to investigate MSCs, and specifically CD45−/CD34−/CD90+ and CD45−/CD34−/CD105+ subpopulations, in the peripheral blood of patients with HCM and to compare the findings with those from healthy individuals. To our knowledge, this is the first time such a study has been conducted.

2. Methods

2.1. Study population

This study included 40 patients with HCM who were referred to our department. HCM was diagnosed by the presence of a nondilated and hypertrophied left ventricle (maximal wall thickness of ≥15 mm) in the absence of another cardiac or systemic disease that might produce LV hypertrophy. We also enrolled 13 subjects with untreated essential hypertension (aged 61±9 years) and with echocardiographic LV hypertrophy [LV mass index (LVMI): men, >115 g/m^2; women, >95 g/m^2] and no indications of other organic heart disease. A full physical examination and routine laboratory tests were performed. Patients with ischemic heart disease, atrial fibrillation or left bundle-branch block on the electrocardiogram, primary valvular heart disease, regional wall-motion abnormalities, or an LV ejection fraction less than 50% on echocardiography were excluded. In addition, we excluded patients with any of the following characteristics: smokers; diabetics; pregnant or lactating women or those potentially childbearing; previous history or medication for hypertension; patients with secondary arterial hypertension; tachyarrhythmia or bradyarrhythmia; cerebrovascular, liver, or renal disease; history of drug or alcohol abuse; any chronic inflammatory or other infectious disease during the last 6 months; thyroid gland disease; body mass index of >40 kg/m^2; or a personal history of anemia, thrombocytopenia, or any other hematological disease. In addition, vascular, metabolic, or neoplastic conditions were ruled out by a careful examination of the history and routine laboratory tests. Height and weight were also measured, and a full echocardiographic examination was performed in all participants.

We also selected a control group of 23 healthy volunteers without symptoms or signs of cardiovascular disease and with no major cardiovascular risk factors, such as diabetes, hypertension, or familiar history of coronary artery disease. This group consisted of patients who came to the emergency department with atypical chest pain but whose clinical and laboratory examinations were normal.

After a rest of 20 min, blood was obtained from all participants, from a superficial brachial vein via a 21-gauge needle, with care to avoid stasis, hemolysis, and contamination by tissue fluids or exposure to glass.

The study complies with the Declaration of Helsinki, it was approved by the hospital's ethics committee, all institutional guidelines were followed, and all participants gave written informed consent.

2.2. Echocardiography study

Standard M-mode and two-dimensional echocardiography was performed using a Vivid 7 (General Electric, Horten, Norway) ultrasound device with a 1.5- to 3.6-MHz wide-angle phased-array transducer, according to the recommendations of the American Society of Echocardiography and the European Association of Echocardiography [13] in order to measure left atrial diameter, LV end-diastolic diameter, LV posterior wall in diastole, interventricular septum in diastole, LV ejection fraction, and relative wall thickness (rWT: septal wall thickness+posterior wall thickness/LV end-diastolic diameter). Pulsed-wave Doppler was used to assess mitral inflow pattern. Peak early velocity (E) and peak late velocity (A) were measured. Color-guided pulsed-wave tissue Doppler imaging from the apical four-chamber view was used to record mitral annulobasal segment motion in the longitudinal axis at a septal site. We measured annular septal and lateral systolic (S), early diastolic (e′), and late diastolic (a) velocities. The individual average for the six measurements was used for analysis. For diastolic assessment, the mitral E wave and tissue Doppler imaging e velocity ratio were also determined. LV mass was calculated according to the Penn convention and expressed as LVMI [14].

2.3. Detection of MSCs by flow cytometry

For each participant, 200 μl of peripheral blood was stained with fluorescence-conjugated mouse antihuman monoclonal antibodies. The antibodies used for staining were CD45−R phycoerythrin-cyanine 7 (PC7), CD34−R phycoerythrin-cyanine 5.1 (PC5), CD90-fluorescein isothiocyanate (FITC), and CD105-R phycoerythrin (PE) (all by Beckman Coulter, Marseille, France). For each sample, an isotype control was prepared in order to monitor background staining using mouse antihuman CD45−PC7 negative control in conjunction with the mouse IgG1 fluorescence-labeled antibodies IgG1-PC5, IgG1-FITC, and IgG1-PE (all by Beckman Coulter, Marseille, France). Staining was performed according to the manufacturer's instructions. Following staining, samples and isotype controls were lysed to remove the red cells and fixed by paraformaldehyde using the Q-prep reagent system (Coulter, Luton, UK). Immediately after fixing, samples and isotype controls were subjected to flow cytometry using the Beckman Coulter Cytomics FC500 apparatus (Beckman Coulter, Inc., Fullerton, CA, USA) and analyzed by the associated CXP software. Approximately 0.5×10^6 cells were passed through the flow cytometer chamber in each experiment. Two populations of MSCs were identified, as cells negative for CD45−PC7 and CD34−PC5 and cells positive for either CD90-FITC or CD105-PE (labeled in text as CD45−/CD34−/CD90+ and as CD45−/CD34−/CD105+). The percentage of MSCs in relation to the total live cells was calculated for each sample and was further normalized by subtracting the percentage of the relevant isotype control.

2.4. Statistical methods

Summary descriptive statistics are presented as mean±S.D. or frequencies (%), as appropriate. Comparisons between the HCM, in hypertensive and control groups, were performed using a two-sided independent samples *t* test. Associations between parameters of interest were assessed by the Pearson correlation coefficient. All comparisons were performed at the two-sided 5% level of significance. The SPSS 20 statistics package was used for the analyses.

3. Results

A total of 40 patients with newly diagnosed HCM participated in the study. Twenty-three healthy individuals with similar age and sex distribution were included as the control group. The baseline clinical characteristics of the participants are presented in Table 1. There were no significant differences in basic clinical and biochemical parameters between the two groups. Thirty-four patients (85%) reported symptoms attributable to HCM (including chest pain, dyspnea, presyncope, and/or syncope). Eight patients (20%) experienced chest pain; 10 patients (25%), syncope or presyncope; and 32 patients (80%), dyspnea on exertion. At the time of enrolment, 16 patients were being treated with β-blockers; 14, with verapamil; and 5, with a combination of the two; 9 patients were also taking diuretics, while 5 patients were not being treated with drugs.