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Relationship between endothelial progenitor cells and vascular endothelial growth factor and its variation with exercise

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ABSTRACT

Background: The aim of our study was to evaluate the effect of programmed physical activity and a single exercise test on the number of $CD309^+$ circulating endothelial progenitor cell (EPC) and their relation to the variation in plasma levels of VEGF in chronic coronary patients.
Methods: 21 patients <75 years with chronic stable coronary artery disease were included. All patients underwent exercise myocardial perfusion SPECT. Then, participants were divided into two groups: one group (11 patients) underwent cardiac rehabilitation program and the other (10 patients) continued with the standard treatment. Blood samples were obtained at baseline, 30 min after exercise ended and at one and three months during follow-up.
Results: VEGF values decreased significantly after exercise SPECT test. After one month, there was a significant increase in VEGF levels compared to those measured immediately after exercise. All patients showed a decrease in the values of EPC at 1 and 3-month follow-up. There was an inverse and statistically significant relation between change of EPC and VEGF between the baseline and 1 month.
Conclusions: The increase of VEGF at 1-month, with respect to baseline values correlated with decreased levels of EPC. This association was independent of the onset of ischemia in the perfusion study.

1. Introduction

The programmed exercise is a very important tool complementary to medical treatment and myocardial revascularization in patients with chronic coronary disease.

One of the proposed mechanisms of action, is that physical activity promotes angiogenesis by increasing the levels and/or half-life of vascular endothelial growth factor (VEGF). Furthermore ischemia is a major stimuli for VEGF production and for local enhancements of its effects [1,2].

It has also been established that endothelial progenitor cells (EPCs) characterized by coexpression of Sca-1 receptor 2 of VEGF or VEGF-2 or KDR [3] improve angiogenesis, promote vascular repair, improve endothelial function, inhibit atherosclerosis and increase the ventricular function after myocardial infarction.

Physical training appears to be the most effective intervention to stimulate EPC [4] in both healthy subjects and patients with coronary disease [5–7].

Exercise increases the production and the number of EPCs in patients with coronary disease and this increase would be time-dependent after a single episode of exercise-induced ischemia [7–9].

However, extreme exercise such as marathon running, seems to decrease the number of circulating EPC defined with $CD34^+$ or $CD133^+$ after the race and even after several days [10,11], while the total number of EPC remains unchanged [11].

The increase in the number and migratory activity generated by exercise could be mediated by an upregulation of NO and VEGF as much as by a reduction in apoptosis of EPC [6].

It has been shown that VEGF is one of the most potent stimuli for the release of EPC [12,13]. VEGF would activate metalloproteinase-9 (MMP-9), which stimulates stem cells to migrate from a quiescent bone marrow niche to the vascular site [14]. The peak of the increase of VEGF clearly precedes the rising levels of EPC [15]. Furthermore, the antigen CD309 is a high affinity receptor for VEGF and plays an important role in hematopoiesis and is also involved in angiogenesis, in embryogenesis

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and in the context of homeostatic and pathological events. CD309-VEGFR-2 has been identified in subsets of hematopoietic stem cells, EPC and mature endothelial cells.

Therefore, the goal of the study was to evaluate in chronic coronary patients, the effect of acute exercise (as the stress test) on EPC and VEGF and its relationships according to the presence of ischemia. A second goal was to study in these patients, the effects of chronic exercise (as rehabilitation program) on the same parameters.

2. Methods

This study included 21 patients, <75 years, with chronic stable coronary artery disease documented by coronary angiography, previous infarction or perfusion tests positive for ischemia performed more than 6 months ago. They should not have participated in groups of programmed physical activity within the last 3 months.

Patients unable to perform physical activity, or with extracardiac conditions affecting survival, associated cardiomyopathies or valvular heart disease, severe ventricular arrhythmia, heart failure, uncontrolled hypertension, FC III–IV angina despite treatment or a positive high-risk exercise stress test were excluded. All patients gave written informed consent to the study protocol, which was approved by the ethical committee.

After baseline evaluation, all the patients underwent exercise stress myocardial perfusion SPECT. According to the test results, two groups were defined:

- Patients with normal perfusion test.
- Patients with positive perfusion test defined by the presence of exercise-induced myocardial ischemia.

Then, the patients were randomly assigned to two groups:

- Patients not undergoing programmed physical activity (control group) (10 patients).
- Patients undergoing rehabilitation program with three exercise bouts weekly over 12 weeks (11 patients).

Aggravation of symptoms, manifestation of events or development of physical disability to continue with the exercise were considered causes of exclusion during follow-up.

2.1. Determination of VEGF

Blood samples were obtained at baseline, 30 min after exercise, and at one and three months during follow-up by vein puncture in tubes containing ethylenediaminetetraacetic acid tripotassium salt (K3EDTA) and stored at −70°.

Determination of VEGF was performed using Quantikine Human VEGF Immunoassay (R&D, catalog # DVE00) that employs the quantitative sandwich enzyme immunoassay technique with a monoclonal antibody specific for VEGF. This technique allows determination of VEGF in a range from 15.6 to 1000 pg/mL. The intra-assay precision and inter-assay precision are 5% and 8%, respectively.

2.2. Determination of EPC

Venous blood samples were collected in tubes containing K3EDTA and preserved at room temperature until they were processed.

Samples were incubated with lysis solution at room temperature for 20 min (2 mL of blood with anticoagulant in 14 mL of lysis liquid), then they were centrifuged (300 × *g*, 10 min), the supernatant was discarded, and the cell button was resuspended in PBS. This procedure was repeated twice in order to obtain a purified erythrocyte-free population of cells.

Sixty microliters of cells were incubated with 20 μL of FcR at room temperature during 15 min. Then they were washed and resuspended in 4 aliquots of 100 μL each:

Aliquot 1: Mouse IgG1-FITC + Mouse IgG1-PE (as negative controls).

Aliquot 2: CD45-FITC + Mouse IgG1-PE.
Aliquot 3: CD45-FITC + CD34-PE.
Aliquot 4: CD34-PE + CD309-PE.

All aliquots were incubated in the dark during 40 min, and afterwards they were diluted with 450 μL of Isoflow and passed through the cytometer.

The samples were acquired on FACSCan-Becton Dickinson equipped with an Argon laser operated at 488 nm and analyzed by flow cytometer with CellQuest software.

CD45-FITC/anti-IgG-PE was used in aliquot 2 to compensate and delimit the $CD45^+$ region used for further acquisitions. So red cells, platelets and cell debris were excluded from analysis.

Region 1 (R1) was obtained from double positivity quadrant ($CD34^+$/$CD45^+$), we took into account cells that also expressed CD34+ from all mononuclear cells ($CD45^+$) (aliquot 3).

Samples of aliquot 4 were acquired to inform the expression of double positivity, and analyzed $CD34^+$/$CD309^+$, using R1 pre-labeled with aliquot 3. CD34 cells that coexpress CD309 support the existence of ECPs.

The amount of ECP is expressed as mean ± standard error (SEM)/100,000 events. The events represent the passage of cells by laser equipment.

2.3. Myocardial perfusion test

Stress-rest SPECT imaging with ^{99m}Tc-sestamibi was performed within the same day, starting either with rest or stress image acquisition.

A cycle ergometer was used for exercise stress test following the Bruce protocol under vital signs control and electrocardiographic monitoring. The test was stopped according to conventional criteria.

The radioisotope was injected at rest and at peak exercise. Images were obtained 30 and 60 min after each injection of the radioisotope, using a step and shoot method in 64 projections. Rest and stress images were acquired in the short-axis view and horizontal and vertical long-axis views. The images were interpreted using a 17-segment heart model. A grading scale of 0 to 4 was used to score perfusion in each segment, where 0 = normal perfusion, 1 = mild hypoperfusion, 2 = moderate hypoperfusion, 3 = severe hypoperfusion, and 4 = absence of perfusion. The sum of the perfusion defects at rest and during stress constituted the summed rest score (SRS) and the summed stress scores (SSS). The summed difference score (SDS) or ischemia was calculated by subtracting the SRS from the SSS. A SDS ≥ 2 was considered positive for ischemia.

2.4. Cardiac rehabilitation program

Physical activity was performed twice a week in a rehabilitation center and once a week at the patient's home. During this period the patients continued with their regular anti-ischemic medication.

Rehabilitation plan consisted in calisthenics, biking with and without workload, gym or recreational activity, and long walks especially designed for each patient.

2.5. Statistical analysis

Categorical variables are presented as frequencies with the corresponding percentage. Continuous variables are expressed as mean ± standard error of the mean (SEM) and median (interquartile range) according to the data distribution. The distribution of quantitative