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Original article

Mobilization of endothelial progenitor cells in acute cardiovascular events in the PROCELL study: Time-course after acute myocardial infarction and stroke

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ABSTRACT

The mobilization pattern and functionality of endothelial progenitor cells after an acute ischemic event remain largely unknown. The aim of our study was to characterize and compare the short- and long-term mobilization of endothelial progenitor cells and circulating endothelial cells after acute myocardial infarction or atherothrombotic stroke, and to determine the relationship between these cell counts and plasma concentrations of vascular cell adhesion molecule (VCAM-1) and Von Willebrand factor (VWF) as surrogate markers of endothelial damage and inflammation. In addition, we assessed whether endothelial progenitor cells behave like functional endothelial cells. We included 150 patients with acute myocardial infarction or atherothrombotic stroke and 145 controls. Endothelial progenitor cells [CD45−, CD34+, KDR+, CD133+], circulating endothelial cells [CD45−, CD146+, CD31+], VWF, and VCAM-1 levels were measured in controls (baseline only) and in patients within 24 h (baseline) and at 7, 30, and 180 days after the event. Myocardial infarction patients had higher counts of endothelial progenitor cells and circulating endothelial cells than the controls (201.0/mL vs. 57.0/mL; $p < 0.01$ and 181.0/mL vs. 62.0/mL; $p < 0.01$). Endothelial progenitor cells peaked at 30 days post-infarction (201.0/mL vs. 369.5/mL; $p < 0.01$), as did VCAM-1 (573.7 ng/mL vs. 701.8 ng/mL; $p < 0.01$). At 180 days post-infarction, circulating endothelial cells and VWF decreased, compared to baseline. In stroke patients, the number of endothelial progenitor cells — but not circulating endothelial cells — was higher than in controls (90.0/mL vs. 37.0/mL; $p = 0.01$; 105.0/mL vs. 71.0/mL; $p = 0.11$). At 30 days after stroke, however, VCAM-1 peaked (628.1/mL vs. 869.1/mL; $p < 0.01$) but there was no significant change in endothelial progenitor cells (90/mL vs. 78/mL; $p < 0.34$). At 180 days after stroke, circulating endothelial cells and VWF decreased, compared to baseline. Cultured endothelial progenitor cells from controls and myocardial infarction patients had endothelial phenotype characteristics and exhibited functional differences in adhesion and Ca^{2+} influx, but not in proliferation and vasculogenesis. In myocardial infarction patients, VCAM-1 levels and mobilization of endothelial progenitor cells peaked at 30 days after the ischemic event. Although a similar VCAM-1 kinetic was observed in stroke patients, endothelial progenitor cells did not increase. Endothelial progenitor cells had mature endothelial capabilities in vitro.

1. Introduction

Acute myocardial infarction (AMI) and atherothrombotic stroke are hallmark examples of endothelial damage complicated by superimposed thrombosis [1,2]. After an endothelial injury, circulating endothelial cells (CECs) are detached from the intimal monolayer [3] and

Abbreviations: AMI, acute myocardial infarction; CECs, circulating endothelial cells; EPCs, endothelial progenitor cells; NIHSS, National Institutes of Health Stroke Scale; NSTEMI, non-ST-segment myocardial infarction; STEMI, ST-segment elevation myocardial infarction; TIA, transient ischemic attack; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor; VWF, Von Willebrand factor.

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endothelial progenitor cells (EPCs) are mobilized from the bone marrow into the peripheral circulation. EPCs can then differentiate into mature endothelial cells, initiating an ideal self-reparative process [4–6].

Increased CEC counts have been described in cardiovascular disease. A higher number of CECs predict adverse events after an acute coronary syndrome [7]. The CEC count has been shown to correlate with other markers of vascular disease such as Von Willebrand factor (VWF) in the acute phase after a myocardial infarction [8]. On the other hand, a reduction in EPCs has been associated with the presence of cardiovascular risk factors [9,10] and with a worse prognosis after ischemic events [11–14]. Systemic inflammation could play a role in the peripheral mobilization of EPCs [15]. Vascular cell adhesion molecule (VCAM)-1 is expressed and upregulated on endothelial cells of atherosclerotic lesions and is a marker of inflammation in atherosclerosis [16].

Information about long-term EPC mobilization and CEC counts after acute ischemic events is scarce because previous studies have been limited to the acute and subacute phase. To our knowledge, no studies have analyzed both cell subtypes after AMI or stroke. In addition, it is unknown if the EPC and CEC counts are correlated with known markers of endothelial injury and activation at different time points. Describing the long-term kinetics of EPC mobilization after two different ischemic events is important to better understand the pathophysiology of endothelial injury and repair.

This study had three objectives: (1) to characterize and compare the short- and long-term mobilization pattern of EPCs and CECs following AMI or stroke; (2) to determine the relationship between cell counts and plasma concentrations of VCAM-1 and VWF at different time points; and (3) to assess whether EPCs behave like functional endothelial cells in terms of cell adhesion, growth curve, vasculogenesis, and intracellular calcium signals triggered by endothelial activators.

2. Methods

The PROCELL study was a multicenter, prospective, population-based, case–control study paired by sex and age. The study protocol was approved by the Institutional Ethics Committees of the three participating hospitals in Spain. All patients gave a written informed consent to participate.

2.1. Study population

Between February 2009 and July 2012, we included 150 patients with AMI or stroke and 145 controls. Controls were recruited from a cross-sectional study nested in the REGICOR cohort study [17]. All included patients participated in follow-up at 7, 30, and 180 days.

2.1.1. AMI patients

We enrolled 100 consecutive patients with AMI. Inclusion criteria were age ≤75 years, with a first AMI and more than one traditional cardiovascular risk factor. Exclusion criteria were previously documented coronary artery disease and established statin therapy, because statins can modify EPC and CEC kinetics. All AMI patients received treatment according to current guidelines, including atorvastatin (40 mg per day at admission, modified during follow-up to achieve <70 mg/dL LDL).

2.1.2. Stroke patients

The study enrolled 50 consecutive patients with acute ischemic stroke or transient ischemic attack (TIA) of atherothrombotic origin. Inclusion criteria were age ≤75 years and initial severity <20 on the National Institutes of Health Stroke Scale (NIHSS). Exclusion criteria were previously documented stroke, disability (modified Rankin scale >2), or established statin therapy, which can modify EPC and CEC kinetics.

All patients with stroke/TIA were admitted to a stroke unit and a vascular neurologist used the NIHSS to categorize three groups of stroke severity (mild < 7, moderate = 7–14, and high < 14). All strokes were assessed by an initial computed tomography scan performed at admission. Control neuroimaging was performed using computed tomography or magnetic resonance imaging. Under current guidelines, all stroke patients received the same treatment as patients with AMI.

2.1.3. Control group

Participants in the REGICOR cohort study [18] who were free of selected cardiovascular risk factors (hypertension, dyslipidemia, diabetes) were invited to participate in this study. Controls were matched by age and sex.

2.2. Endothelial progenitor cell and circulating endothelial cell counts

Blood samples were collected into low-molecular-weight heparin tubes and processed twice within 4 h of extraction. Circulating EPC and CEC counts were determined by flow cytometry (FC5000 cytometer, Beckman–Coulter, Madrid, Spain). EPCs were defined as negative for CD45 and positive for CD34, KDR, and CD133 [CD45 − CD34 + KDR + CD133 +]. CECs were defined as negative for CD45 and positive for CD146 and CD31 [CD45 − CD146 + CD31 +], as previously described [19]. Counts were calculated by multiplying the ratio of EPCs and CECs obtained in the flow cytometry analysis by the number of leukocytes/mL in the blood sample to obtain the absolute number of EPCs and CECs per 1 mL of whole blood.

2.3. Endothelial progenitor cell culture and characterization

Peripheral blood samples were collected from controls upon inclusion and from AMI patients within 24 h of the event. Blood was recovered in heparinized tubes and processed within 2 h. Unfortunately, the hospital from which stroke patients were recruited was too far from the lab to process the samples within this time limit; in this group, therefore, EPCs were not isolated and cultured in vitro. Mononuclear cells were isolated by density gradient centrifugation. After isolation, mononuclear cells were seeded on fibronectin-treated culture dishes. After 24 h, nonadhered cells were removed and attached cells were further cultured up to 30 days, as previously described [20].

2.4. Functional characterization of cultured endothelial progenitor cells

2.4.1. Cell adhesion

EPC adhesion was defined as the cells' ability to adhere to a fibronectin matrix. Two independent observers counted adhered cells in 6 random squares. Data were expressed as percentage of adhered cells related to total number of seeded cells.

2.4.2. Cell proliferation

Cell-cycle phases were determined by propidium iodide staining to determine cell DNA content and flow cytometry. To determine the proliferative response to the growth factors contained in EGM-2 culture media, confluent EPC cultures were starved for 48 h, stimulated for 18 h, and then proliferative cells were counted. The EPCs were recovered, fixed, and stained with propidium iodide-RNAse solution (Immunostep, Salamanca, Spain) for 15 min. Stained cells were analyzed with FC500 cytometer and Infinicyt software (Cytognos, Salamanca, Spain).

2.4.3. Growth curve

To determine the expansion capacity over time, EPCs were seeded on 24-well plates (1.5×10^4 cells/well), and media was changed daily. For 6 days, cells were harvested daily, pelleted, and re-suspended in Trypan blue solution (Sigma Aldrich). Viable EPCs were determined by counting cells in a modified Neubauer chamber.

2.4.4. Vasculogenesis

The ability of EPCs to form capillary-like structures was determined by seeding 1.5×10^5 EPCs on Matrigel-pretreated plates. Total length of capillary-like structures is expressed in micrometers.