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Cardiomyogenic differentiation potential of human endothelial progenitor cells isolated from patients with myocardial infarction

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Abstract

Background aims. Endothelial progenitor cells (EPCs) are known to play a beneficial role by promoting postnatal vasculogenesis in pathological events, such as ischemic heart disease and peripheral artery disease. However, little is known about the potential of EPCs to restore heart damage tissue. We compared the cardiac differentiation capacity of EPCs isolated from peripheral blood of patients with acute myocardial infarction (AMI) with EPCs obtained from umbilical cord blood (UCB). *Methods.* EPCs from both origins were isolated by density gradient centrifugation and characterized through the use of endothelial markers (UEA-1lectin, CD133 and KDR) and endothelial cell colony-forming unit assay. Cardiac differentiation capacity of EPCs was assessed by immunofluorescence and reverse transcriptase–polymerase chain reaction after 5-azacytidine (5-aza) induction. *Results.* No significant differences were observed between the number of endothelial cell colony-forming units in peripheral blood of patients with AMI and samples from UCB. Moreover, 5-aza induced the appearance of myotube-like structures and the positive expression of sarcomeric α-actinin, cardiac troponin I and T and desmin in a similar pattern for both cell sources, which indicates a comparable acquisition of a cardiac-like phenotype. *Conclusions.* For the first time, we have compared, *in vitro*, the cardiomyogenic potential of EPCs derived from patients with AMI with UCB-derived EPCs. Our data indicate that EPCs obtained from both origins have similar plasticity and functions and suggest a potential therapeutic efficacy in cardiac cell therapy.

Key Words: *acute myocardial infarction, cardiomyocyte differentiation, endothelial progenitor cells, umbilical cord blood*

Introduction

Myocardial infarction is the result of coronary artery obstruction with the consequent reduction of blood supply to the heart muscle and the massive loss of cardiomyocytes that are replaced by a non-functional scar tissue (1).

In 1997, Asahara *et al.* (2) identified for the first time $CD34^+$ circulating bone marrow (BM)-derived cells, implicated in physiological or pathological neovascularization, that were identified as endothelial progenitor cells (EPC). These blood cells were characterized by the expression of several markers such as CD133, CD34, KDR or VE-cadherin (3). However, controversy regarding overlapping expression of markers by EPCs and other hematopoietic progenitor cells has emerged (4). In fact, EPCs have been subdivided into two main categories (i) hematopoietic or (ii) non-hematopoietic EPCs. The hematopoietic EPCs are a heterogeneous cell population, which include colony-forming EPCs, non–colony-forming ''differentiating'' EPCs, myeloid EPCs and angiogenic cells (5). These progenitor cells,

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regardless their origin, have the potential to differentiate into mature endothelial cells (ECs) and to play a significant role contributing to neovascularization in ischemic tissues (2,6,7). Therefore, the significant increase of progenitor cells after myocardial infarction (3,8–10), indicates that circulating EPCs could represent a useful marker of pathogenesis and prognosis of cardiovascular diseases (11–15).

The angiogenic properties of EPCs, isolated from circulating peripheral blood (PB) and umbilical cord blood (UCB), have been proven in both experimental and *in vivo* settings (16,17). Recent studies in animal models of ischemic cardiomyopathy have suggested that transplanted EPCs could improve heart recovery after injury (18–20). Furthermore, ongoing clinical trials are using EPC-enriched cell populations with the aim to elucidate the therapeutic effects of these progenitor cells on ischemic diseases (21). Although initial results from clinical trials assessing the safety and feasibility of autologous progenitor cell transplantation are promising, a long-term follow-up of these patients must be evaluated.

On the other hand, it has been suggested that EPCs isolated from mouse BM and from human PB could differentiate into cardiomyocytes under certain *in vitro* conditions (22–24). On the basis of this potential plasticity, additional studies are still needed to elucidate the possible beneficial effect of circulating EPCs from patients who have had an infarct in the treatment of ischemic diseases. In the present study, we tested the cardiac differentiation potential of circulating EPCs from PB of patients who had been diagnosed with acute myocardial infarction (AMI) and compared the cells with EPCs from UCB.

Methods

Patients

We studied 24 patients, admitted with a diagnosis of AMI, defined as: (i) an acute coronary syndrome, with ST-elevation myocardial infarction (STEMI) with suggestive chest pain, (ii) an elevation of at least 3 mm in the ST segment in at least three precordial leads and (iii) within 8 h of symptoms. In addition, the specific biomarker cardiac troponin T was measured in the patient's serum, showing a positive troponin T peak (54.4 ± 13.1 ng/mL). Patient mean age was 59.5 ± 12.4 years; men represent 72% of the patients included in the study. Furthermore, hypertension was present in the 66% and hypercholesterolemia in 44% of the diagnosed patients. Finally, average ejection fraction was 51.9% ± 12.2%. Pre-infarction angina was defined as the presence of at least one chest pain lasting <30 min the week before the onset of the infarction. Patients were excluded if they had had chest pain compatible with angina for more than 1 week before the infarction or if they had underlying structural heart disease (cardiomyopathy or important valve disorders). Moreover, patients with a history of rheumatoid arthritis, hepatic, hematologic, or coagulation disorders, cancer or other acute or chronic inflammatory diseases such diabetes mellitus were not included in the study. Informed consent was obtained from all patients, and heparinized blood samples (20 mL) were drawn from all subjects. Human UCB samples ($n = 25$, 20 mL) were obtained from the Centro Regional de Transfusión Sanguínea y Tejidos de Málaga, Málaga, Spain, according to institutional guidelines. Samples were generally processed within 24–48 h of collection. Myocardial tissue samples were obtained from forensic cadaver autopsies no more than 12 h postmortem as described previously (25).

Quantification of endothelial markers in mononuclear cells

Mononuclear cells (MNCs) isolated by density gradient centrifugation (Histopaque-1077, Sigma, St Louis, MO, USA) from PB of patients with AMI and from UCB were washed and resuspended in phosphate-buffered saline (PBS) with 2% bovine serum albumin (Sigma) and 2 mmol/L ethylene diamine tetra acetic acid (Sigma). Cells were incubated in the dark at 4°C for 45 min with the following fluorochrome-conjugated monoclonal antibodies: CD133-PE (Miltenyi Biotec, Auburn, CA, USA), KDR-APC (R&D Systems, Minneapolis, MN, USA), CD34–fluorescein isothiocyanate (FITC) and CXCR4-APC (BD Biosciences, San Jose, CA, USA). Cells were then washed in PBS and analyzed in a fluorescence-activated cell sorting (FACS) Canto II cytometer equipped with the FACS Diva analysis software (BD Biosciences). Data obtained are expressed as mean ± standard error from four independent experiments performed in triplicate ($P < 0.05$).

Isolation and culture of EPCs

Samples of PB from patients with AMI and from UCB (20 mL) were processed by density-gradient (Histopaque-10771, Sigma) for 25 min at 1500 rpm/min and washed three times in PBS with 2% fetal bovine serum (FBS). Cells were further processed as described before (26). Briefly, 5×10^6 isolated MNCs per well were plated on fibronectin-coated six-well dishes (BD Biosciences) in endothelial cell basal medium, EBM-2 (Lonza, Basel, Switzerland),