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Short communication

Are endothelial progenitor cells mobilized by myocardial ischemia or myocardial necrosis? A cardiac magnetic resonance study

Italo Porto[a,*], Antonio Maria Leone[a], Giovanni Luigi De Maria[a], Christian Hamilton Craig[a,c], Alessandra Tritarelli[a], Claudia Camaioni[a], Luigi Natale[b], Giampaolo Niccoli[a], Luigi M. Biasucci[a], Filippo Crea[a]

[a] Department of Cardiovascular Medicine, Catholic University of the Sacred Heart, Largo A. Gemelli, 8, 00168 Rome, Italy
[b] Department of BioImaging, Catholic University of the Sacred Heart, Rome, Italy
[c] University of Queensland, Brisbane, Australia

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ABSTRACT

Background: In ST-elevation myocardial infarction (STEMI) patients, the main stimuli involved in endothelial progenitor cells (EPCs) mobilization are not fully understood. We aimed to assess by cardiac magnetic resonance (CMR) whether the extent of ischemic myocardium (area at risk (AAR)) or of necrotic myocardium (infarct size (IS)) can be correlated to levels of circulating EPCs.
Methods: Peripheral EPCs were measured in fifteen STEMI patients at 24 h after successful primary percutaneous coronary intervention (pPCI). Between two and four days after pPCI all patients underwent CMR assessment of myocardial AAR, IS, myocardial salvage (MS) and microvascular obstruction at late gadolinium enhancement CMR (LG-MVO).
Results: CD34+/KDR+, CD34+/KDR+/CD45dim, CD34+/KDR+/CD45−, EPCs were related to extent of AAR (rho = 0.51, $p = 0.05$; rho = 0.55, $p = 0.03$; rho = 0.72, $p = 0.002$, respectively), while no relationships were detected with IS, MS or LG-MVO.
Conclusions: Our data show that EPCs were strongly correlated to extent of myocardial AAR, thus suggesting that progenitor cells mobilization in STEMI develops in response to myocardial ischemia and not to myocardial necrosis.

1. Introduction

Circulating progenitor cells (CPCs) are bone marrow derived elements with reparative and regenerative properties known to be mobilized in patients with myocardial infarction (MI) [1], and related to improved prognosis in patients with cardiovascular diseases [2]. Endothelial progenitor cells (EPCs) are a subtype of CPC, demonstrated to contribute to repair injured endothelium [3]. While uncertainties still exist regarding which combination of surface markers should be used for the cytofluorimetric detection of "true" EPC, some authors maintain that CD34+/KDR+/CD45− cells might represent the best compromise [4]. In previous studies, levels of both CPC and EPC in peripheral blood were shown to progressively increase after an MI, peaking at 5–7 days, and then decreasing to basal levels [1,5,6]. Moreover, persistently raised levels of CPC and EPC were associated to favorable ventricular remodeling and better prognosis after MI [1,5,6]. However, debate still exists about the main trigger responsible for EPC mobilization.

We aimed to assess by cardiac magnetic resonance (CMR) whether the extent of ischemic myocardium, corresponding to the area at risk (AAR), or the extent of necrotic myocardium, corresponding to infarct size (IS), can be correlated to the levels of circulating CPC and EPC in the acute phase after MI.

2. Methods

2.1. Patient population and biochemistry

In this small, observational, prospective study, 15 consecutive patients from a total of 62 consecutive patients admitted with STEMI to our Coronary Care Unit (CCU) from December 2009 to June

Abbreviations: AAR, area at risk; CAD, coronary artery disease; CK–MB, creatin-kinase–MB; CMR, cardiac magnetic resonance; CPC, circulating progenitor cells; dim, diminished; EPC, endothelial progenitor cells; hs-CRP, high-sensitivity CRP; LGE, late gadolinium enhancement; LG-MVO, microvascular obstruction at late gadolinium enhancement; mAbs, monoclonal antibodies; MI, myocardial infarction; MVO, microvascular obstruction; IS, infarct size; ISmass, infarct size as mass; IS %, infarct size as percentage; pPCI, primary percutaneous coronary intervention; STEMI, ST elevation myocardial infarction.

* Corresponding author. Tel.: +39 06 30 15 41 87; fax: +39 06 30 55 535.
E-mail addresses: italo.porto@gmail.com, i.porto@eudoramail.com, i.porto@lycos.com (I. Porto).

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2010 and undergoing primary percutaneous coronary intervention (pPCI), were enrolled (Supplementary Fig. 1).

All patients were admitted to our CCU with chest pain, new ST-segment elevation at the J-point in two contiguous leads of at least 2 mm in men and 1.5 mm in woman on electrocardiogram and cardiac Troponin T (TnT) elevation [7].

Exclusion criteria were age >80 years, ejection fraction <30%, Killip class IV, prior MI or major surgical interventions in the previous three months, MI secondary to ischemia from imbalance of oxygen supply and demand (type 3 MI), recent or chronic infective or inflammatory disease, malignancy, contraindication to CMR examination.

All patients were treated with aspirin (250 mg i.v., then 75 mg daily) and clopidogrel (loading dose of 600 mg or 300 mg if already on clopidogrel, then 75 mg daily), plus additional standard antithrombotic therapy with heparin (standard or low molecular weight). Glycoprotein IIb/IIIa antagonists were administered at physician discretion. Cardiac TnT was measured every 6 h, from admission up to a peak value, and then every 12 h, by the acute chemical laboratory at Gemelli Hospital using a 1-step electroimmunoassay by electrochemiluminescence technology (Elecsys 2010, Roche), with a lower detection limit of 0.01 mg/L. Coded plasma samples were stored at −80 °C and high-sensitivity CRP (hs-CRP) was assayed in a latex-enhanced immunonephelometric high-sensitivity assay (Dade Behring, Marburg, Germany).

Local ethics committee approved the study and all patients enrolled signed informed consent. All procedures were followed in accordance with the ethical standards of the responsible committees on human experimentation and with the Helsinki Declaration.

2.2. CPC and EPC analysis

Peripheral venous blood samples were collected within 24 h after catheterization and at 2 months follow up (range 1–4). Samples were kept at room temperature and analyzed within 2 h. 100 μl of EDTA-anticoagulated peripheral blood was incubated for 15 min in the dark with 5 μl of the following mAbs (monoclonal antibodies): CD34-FITC (Beckman Coulter, Miami, USA), CD45-PC5 (Beckman Coulter, Miami, USA) and/or VEGF R2-PE (KDR; R&D Systems, Minneapolis, MN, USA) and/or CD133/1-PE (Miltenyi Biotec, Auburn, CA, USA). Appropriate fluorochrome-conjugated isotype-matched mAb purchased from different manufacturers was used as control for background staining.

After incubation, cells were processed with Immuno-Prep reagent system (Beckman Coulter, Miami, USA) using Coulter Q-prep (Beckman Coulter, Miami, USA) and then the samples were run through an EPICS XL (Beckman Coulter, Miami, USA). EPCs were defined as CD34+/KDR+, CD34+/KDR+/CD45−, CD34+/KDR+/CD45dim cells, while CD34+/CD133+, CD34+/KDR+/CD45+, CD34+/CD133/CD45+ and CD34+/CD133+/CD45− cells were all considered different CPC subpopulations [8]. CPC and EPC were expressed as absolute percentage of cells per total number of cytometric events. Technical details of cytofluorimetric analysis are provided in Supplementary Material.

2.3. CMR analysis

All patients underwent CMR 2–4 days after pPCI, on a 1.5-T scanner (Signa Excite II, GE Medical Systems, Buc, Paris). For more technical details, see Supplementary Materials. Briefly, IS was quantified using a signal threshold of >5-standard deviations from normal myocardium, and expressed in grams (ISmass) and as a normalized percentage of left ventricular mass (IS%). Delayed-enhancement-MVO (LG-MVO) volume was quantified using a manual region-of-interest to trace areas of low signal within the brightly enhancing infarcted myocardium, and expressed in grams (LG-MVOmass) and normalized to left ventricular mass (LG-MVO%).

Myocardial edema, representing the area-at-risk (AAR) was evaluated using T2-weighted short tau-inversion recovery (STIR) images, using a signal intensity threshold >2 standard deviations above the mean signal of remote non-infarcted myocardium, using MASS software (Medis, Leiden, The Netherlands). Slow-flow artifact was carefully excluded by comparison with SSFP images. AAR was quantitatively expressed in grams and as percentage of total LV mass, as previously described [9]. Myocardial salvage index (MSI) was calculated as the difference between the AAR on T2W images and the IS on delayed enhancement-CMR images divided by the AAR, expressed as % of the AAR.

Table 1
Clinical characteristics of the studied sample.

Clinical Characteristics	
Male (n/%)	14 (93.3)
Age (mean ± SD)	60.2 ± 7.8
Diabetes Mellitus (n/%)	3 (20.0)
Hypertension (n/%)	10 (66.7)
Smoking (n/%)	6 (40.0)
Family History (n/%)	3 (20.0)
Hypercholesterolemia (n/%)	10 (66.7)
Total Cholesterol (mg/dl) (median IQR)	167 (153–177)
LDL (mg/dl) (median IQR)	97 (89–132)
HDL (mg/dl) (median IQR)	40 (34–45)
Triglycerides (mg/dl) (median IQR)	117 (73–148)
Statin therapy before admission (n/%)	3 (20)
Ejection Fraction (%) (median IQR)	50 (46.5–52.7)
Killip Class on admission	
I (n/%)	6 (40.0)
II (n/%)	6 (40.0)
III (n/%)	3 (20.0)
Total Ischemic Time	
≤ 3h (n/%)	4 (26.7)
3 - 6h (n/%)	6 (40.0)
≥ 6h (n/%)	5 (33.3)
Serum Creatinin (mg/dl) (median IQR)	1.1 (0.9–1.2)
Troponin on admission (ng/ml) (median IQR)	0.17 (0.03–2.65)
Troponin peak (ng/ml) (median IQR)	7.14 (4.00–13.01)
Culprit vessel	
LAD (n/%)	10 (66.7)
LCx (n/%)	0 (0)
RCA (n/%)	5 (33.3)
Number of diseased vessels	
1 (n/%)	6 (40.0)
2 (n/%)	6 (40.0)
3 (n/%)	3 (20.0)
Thrombus aspiration (n/%)	14 (93.3)
GPIIb/IIIa antagonists (n/%)	7 (46.7)
Direct Stenting (n/%)	8 (53.3)
BMS (n/%)	12 (80.0)
DES (n/%)	2 (13.3)
POBA (n/%)	1 (6.7)
CMR measures	
EDV (ml) (median IQR)	161 (151–175)
ESV (ml) (median IQR)	83 (70–99)
Ejection Fraction (%) (median IQR)	50 (39–55)
AAR % (median IQR)	29 (24.4–41.3)
IS mass (grams) (median IQR)	38.3 (15.8–48.3)
IS % (median IQR)	19.1 (11.9–26.1)
Myocardial Salvage % (median IQR)	28.3 (21.0–42.6)
LG-MVO mass (grams) (median IQR)	0 (0–4.8)
LG-MVO % (median IQR)	0 (0–3.0)

2.4. Statistical analysis

All variables were expressed as mean ± standard deviation or as median with interquartile range (IQR), as appropriate. Main results (EPC count, AAR, ISmass, IS%, LG-MVOmass, LG-MVO%, MSI) are