

Mobilization of bone marrow-derived progenitor cells in acute coronary syndromes

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Abstract: Two hypotheses explain the role of adult progenitor cells in myocardial regeneration. Stem cell plasticity which involves mobilization of stem cells from the bone marrow and other niches, homing to the area of tissue injury and transdifferentiation into functional cardiomyocytes. Alternative hypothesis is based on the observations that bone marrow harbors a heterogenous population of cells positive for CXCR4 - receptor for chemokine SDF-1. This population of non-hematopoietic cells expresses genes specific for early muscle, myocardial and endothelial progenitor cells (EPC). These tissue-committed stem cells circulate in the peripheral blood at low numbers and can be mobilized by hematopoietic cytokines in the setting of myocardial ischemia. Endothelial precursors capable of transforming into mature, functional endothelial cells are present in the pool of peripheral mononuclear cells in circulation. Their number significantly increases in acute myocardial infarction (AMI) with subsequent decrease after 1 month, as well as in patients with unstable angina in comparison to stable coronary heart disease (CHD). There are numerous physiological and pathological stimuli which influence the number of circulating EPC such as regular physical activity, medications (statins, PPAR- γ agonists, estrogens), as well as numerous inflammatory and hematopoietic cytokines. Mobilization of stem cells in AMI involves not only the endothelial progenitors but also hematopoietic, non-hematopoietic stem cells and most probably the mesenchymal cells. In healthy subjects and patients with stable CHD, small number of circulating CD34⁺, CXCR4⁺, CD117⁺, c-met⁺ and CD34/CD117⁺ stem cells can be detected. In patients with AMI, a significant increase in CD34⁺/CXCR4⁺, CD117⁺, c-met⁺ and CD34/CD117⁺ stem cell number in the peripheral blood was demonstrated with parallel increase in mRNA expression for early cardiac, muscle and endothelial markers in peripheral blood mononuclear cells. The maximum number of stem cells was found early in ST-segment elevation myocardial infarction (<12 hours) with subsequent decrease through the 7-day follow-up and with concomitant changes in the levels of cytokines involved in the inflammatory response and stem cell recruitment. Moreover, peak expression of cardiac muscle and endothelial markers occurred at the same time as the most significant increase in CD34/CXCR4⁺ stem cell number. The SDF-1/CXCR-4 axis seems particularly important in stem/muscle progenitor cell homing, chemotaxis, engraftment and retention in ischaemic myocardium. The significance of autologous stem cells mobilization in terms of cardiac salvage and regeneration needs to be proved in humans but it seems to be a reparative mechanism triggered early in the course of acute coronary syndromes.

Key words: Endothelial progenitor cells - Tissue committed stem cells - Myocardial infarction

Introduction

Numerous animal models of acute myocardial infarction as well as clinical trials involving intracoronary infusion of bone marrow and peripheral blood-derived autologous progenitor cells have documented that these cells may have the potential to regenerate or/and improve function of the myocardium after the ischaemic injury [3, 4, 17].

Two hypotheses explain the role of adult progenitor cells in tissue regeneration. Stem cell plasticity involves

the increase of hematopoietic cytokines with subsequent mobilization of stem cells from the bone marrow and other niches (*e.g.* skeletal muscles), homing to the area of tissue injury and transdifferentiation into functional cardiomyocytes [11]. Alternative hypothesis was formulated, which is based on the observations that bone marrow harbors a heterogenous population of cells positive for CXCR4 - receptor for chemokine stromal cell-derived factor-1 (SDF-1). This population of non-hematopoietic cells expresses genes specific for

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early muscle, myocardial and endothelial progenitor cells [10]. These tissue-committed stem cells (TCSC) circulate in the peripheral blood at low numbers and can be mobilized by hematopoietic cytokines in the setting of vascular injury, ischemia and after cytokine stimulation [9, 20]. Evidence that migratory cells participate in the formation of virtually all cardiac structures in transplanted heart comes from work of Quaini *et al.* documenting the systemic chimerism in cases in which a male patient received a heart from a female [14]. The significance of autologous stem cell mobilization in terms of cardiac salvage and regeneration needs to be proved in humans but it seems to be a reparative mechanism triggered early in the course of acute coronary syndromes (ACS).

Mobilization of endothelial progenitor cells (EPC)

Endothelial precursors capable of transforming into mature, functional endothelial cells are present in the pool of peripheral mononuclear cells in circulation [1]. Shintani *et al.* [16] described for the first time a significant increase in CD34⁺ peripheral blood mononuclear cells (PBMNC) positive for lineage markers (KDR, VE-cadherin, CD31) as well as function (DiI-acLDL uptake) and morphology of EPC. The number of EPC peaked after 1 week after acute myocardial infarction (AMI) with subsequent decrease after 1 month. Conversely, according to Masa *et al.* [13], the peak mobilization of EPC occurs early (hours) in AMI. These findings were confirmed by George and coworkers by assessing of the EPC number in patients with unstable angina in comparison to stable coronary heart disease (CHD). They demonstrated increased number of circulating EPC with typical immunophenotype and functional properties in patients with unstable angina. In 3 months-follow-up, the number of EPCs was reduced by 50% in comparison to baseline counts. Moreover, a positive correlation was found between systemic C-reactive protein levels and circulating EPC [5]. Both studies give an important message because recent data from both experimental and clinical studies show that EPC can potentially be used for therapeutic angiogenesis resulting in the improvement of myocardial function. The spontaneous mobilization of these cells in AMI and unstable angina may be an important reparatory mechanism [1, 5].

There are numerous physiological and pathological stimuli which influence the number of circulating EPC, as shown in Table 1.

Mobilization of tissue-committed stem cells

Mobilization of stem cells in AMI involves not only the endothelial progenitors but also hematopoietic, non-hematopoietic stem cells and most probably the mesenchy-

Table 1. Factors influencing the mobilization of bone marrow progenitor cells [9,14-20]

Factor	Change in cpc number/function
Physical activity	Increase
Age	Decrease with age
CHD risk factors	Decrease with number of risk factors
Limb ischemia	Increase
Acute myocardial infarction/unstable angina	Increase
Vascular trauma (<i>eg.</i> balloon dilatation)	Increase
Atrial fibrillation	Increase
HMG-CoA reductase inhibitors	Increase; augmented incorporation into neovascularization foci in cornea, improve EPC-associated neovascularization in mice with AMI
Estradiol PPAR- γ agonists	Increase Increase; improve endothelialization
VEGF	Increase
Erythropoietin	Increase
Angiopoietin-1	Increase
G-CSF, GM-CSF	Increase
SDF-1	Increase
HGF	Increase
LIF	Increase

PPAR- γ - peroxisome proliferator-activated receptor gamma; SDF-1 - stromal cell-derived factor-1; HGF - hepatocyte growth factor; LIF - leukemia inhibitory factor

mal cells [4, 9, 20]. In healthy subjects and patients with stable CHD, small number of circulating CD34⁺, CXCR4⁺, CD117⁺, c-met⁺ and CD34/CD117⁺ stem cells can be detected. In patients with AMI, a significant increase in CD34⁺/CXCR4⁺, CD117⁺, c-met⁺ and CD34/CD117⁺ stem cell number in peripheral blood was demonstrated with parallel increase in mRNA expression for early cardiac, muscle and endothelial markers in PBMNC. The maximum number of stem cells was found early in ST-segment elevation myocardial infarction (STEMI, <12 hours) with subsequent decrease through the 7-day follow-up with concomitant changes in the levels of cytokines involved in the inflammatory response and stem cell recruitment. Moreover, peak expression of cardiac muscle and endothelial markers in PBMNC occurred at the same time as the most significant increase in CD34/CXCR4⁺ stem cell number [20].

Massa *et al.* [13] demonstrated approx. 6 hours from the onset of AMI a significant 5.8-fold increase in CD34⁺ (both % of leukocytes and absolute number of cells) mononuclear cells including both hematopoietic

progenitor and endothelial progenitor cells which gradually declined to baseline within 2 months in comparison to patients with stable angina and healthy controls.

Since these studies demonstrated a significant increase in circulating hematopoietic and endothelial progenitor cells peaking early - already a few hours after the onset of symptoms of AMI, it seems that this phenomenon is independent of the medical treatment and invasive interventions the patients received.

The SDF-1/CXCR-4 axis seems particularly important in stem/muscle progenitor cell homing, chemotaxis, engraftment and retention in ischaemic myocardium. Kucia *et al.* [9] showed that murine bone marrow contains pool of cells that express early cardiac lineage markers (Nkx2.5/Csx, GATA-4, and MEF2C) and can be mobilized in experimental myocardial infarction. Flow cytometry analysis revealed that cells expressing these markers are nonadherent, nonhematopoietic cells of the following immunophenotype: CXCR4⁺/Sca-1⁺/lin⁻/CD45 in mice and CXCR4⁺/CD34⁺/AC133⁺/CD45⁻ in humans. These cells are mobilized into the peripheral blood after myocardial infarction and chemoattracted to the infarcted myocardium by SDF-1/CXCR4, HGF/c-met and LIF-LIF-R (leukemia inhibitory factor/ leukemia inhibitory factor receptor) axes [9]. This is the first proof that the postnatal bone marrow contains nonhematopoietic population of cells that express markers for cardiac differentiation [9-11].

Mobilization of stem cells in relation to clinical and laboratory parameters

In patients with STEMI, low left ventricular ejection fraction (LVEF) and high NT-proBNP levels (both independent predictors of clinical outcome), the mobilization of stem cells expressing CD34, CXCR4 and c-met antigens is significantly lower in comparison to patients with LVEF > 40% and low levels of NT-proBNP. Moreover, the number of mobilized stem cells was negatively correlated with cardiac necrosis markers (troponin I, CK-MB) [19].

Further questions

Despite of huge progress in understanding of bone marrow-derived cell (BMC) mobilization, much more questions remain to answered, *e.g.* is the mobilization of BMC in acute coronary syndromes the reparatory response leading to a significant myocardial salvage or just a nonspecific inflammatory reaction? Furthermore, so far no large clinical trials showed benefits from mobilization of EPC using exogenous cytokines in patients with AMI.

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