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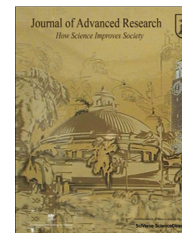

ORIGINAL ARTICLE

Endothelial progenitor cells regenerate infracted myocardium with neovascularisation development☆

M.T. Abd El Aziz [a], E.A. Abd El Nabi [a,b], M. Abd El Hamid [c], D. Sabry [a,*], H.M. Atta [a,d], L.A. Rahed [a], A. Shamaa [e], S. Mahfouz [f], F.M. Taha [a], S. Elrefaay [g], D.M. Gharib [a], Khaled A. Elsetohy [h]

[a] Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Cairo University, Cairo, Egypt
[b] Clinical Biochemistry Department, Faculty of Medicine, King Abdulaziz University, North Jedda, Saudi Arabia
[c] Cardiology Department, Faculty of Medicine, Cairo University, Cairo, Egypt
[d] Clinical Biochemistry Department, Faculty of Medicine, King Abdulaziz University, Rabigh branch, Jeddah, Saudi Arabia
[e] Surgery Department, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
[f] Pathology Department, Faculty of Medicine, Cairo University, Cairo, Egypt
[g] Nuclear Medicine Department, Faculty of Medicine, Cairo University, Cairo, Egypt
[h] Obstetrics and Gynaecology Department, Faculty of Medicine, Cairo University, Cairo, Egypt

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ABSTRACT

We achieved possibility of isolation, characterization human umbilical cord blood endothelial progenitor cells (EPCs), examination potency of EPCs to form new blood vessels and differentiation into cardiomyoctes in canines with acute myocardial infarction (AMI). EPCs were separated and cultured from umbilical cord blood. Their phenotypes were confirmed by uptake of

Abbreviations: CTO, chronic total occlusion; CAG, coronary angiography; AMI, acute myocardial infarction; DILDL-FITC labeled UEA-11, 1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine-labeled acetylated LDL (DiLDL,) and FITC-labeled Ulex europaeus agglutinin-1; MVD, multivessel disease; CFU, colony forming unit.

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* Corresponding author. Tel.: +20 01111200200.
E-mail addresses: dinnasabry69@yahoo.com, dinasabry@kasralainy.edu.eg (D. Sabry).

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double stains dioctadecyl tetramethylindocarbocyanine-labeled acetylated LDL and FITC-labeled Ulex europaeus agglutinin 1 (DILDL-UEA-1). EPCs of cord blood were counted. Human VEGFR-2 and eNOS from the cultured EPCs were assessed by qPCR. Human EPCs was transplanted intramyocardially in canines with AMI. ECG and cardiac enzymes (CK-MB and Troponin I) were measured to assess severity of cellular damage. Histopathology was done to assess neovascularisation. Immunostaining was done to detect EPCs transdifferentiation into cardiomyocytes in peri-infarct cardiac tissue. qPCR for human genes (hVEGFR-2, and eNOS) was done to assess homing and angiogenic function of transplanted EPCs. Cultured human cord blood exhibited an increased number of EPCs and significant high expression of hVEGFR-2 and eNOS genes in the culture cells. Histopathology showed increased neovascularization and immunostaining showed presence of EPCs newly differentiated into cardiomyocyte-like cells. Our findings suggested that hEPCs can mediate angiogenesis and differentiate into cardiomyoctes in canines with AMI.
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Introduction

Chronic total occlusion (CTO) is diagnosed in patients with coronary artery disease during angiography [1]. Multivessel disease (MVD) effects are due to the presence of CTO in a noninfarct-related artery [2]. CTO lesion in a non-infarct related artery was a high risk factor for mortality after acute myocardial infarction (AMI) [3].

Endothelial progenitor cells (EPCs), described as a heterogeneous population of circulating cells in peripheral blood [4]. Their origin is found in multiple precursors, such as hemangioblasts, non-hematopoietic precursors, monocytic cells, or tissue-resident stem cells. EPCs play an important role in vasculogenesis because of their capacity to proliferate, migrate, differentiate *in vivo* and *in vitro* into endothelial cells, and incorporate into the preexisting endothelium. Thus, phenotypically, they have morphofunctional characteristics of both hematopoietic and mature endothelial cells [5]. EPCs are rare, representing approximately 0.01%–0.0001% of the mononuclear fraction in peripheral blood. However, several stimuli, such as physical exercise, can mobilize them from bone marrow, temporarily increasing their number in peripheral circulation. EPCs neovasculogenesis function was due to secretion of pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and granulocyte colony stimulating factor (G-CSF) [6]. Early EPCs (present in the BM or directly after reaching the bloodstream) are CD133+/CD34+/VEGFR2+ cells, whereas circulating EPCs are $CD34^{+}$ and VEGFR-2+, $CD133^{-}$ and start to express membrane molecules typical to mature ECs [7].

Experimental studies revealed that intravascular or intramyocardial administration of EPCs may enhance functional regeneration of infracted myocardium and neovascularization of ischemic myocardium [8]. Clinical studies suggested that intracoronary infusion of progenitor cells is accessible and may greatly affect left ventricular contractile function or decrease infarct size in patients with AMI. Previous results either experimental or clinical provide important evidence about use of progenitor cell in cell therapy of chronic coronary artery disease [9].

Studies showed an increase in capillary density associated with an improvement of ventricular function and a reduction in ventricular size three months after stem cell transplantation into the under perfused myocardial segments compared to control group [10,11]. These effects could be increased by preincubation of the stem cells with cardiomyogenic growth factors leading to a cardiomyogenic differentiation. Applying these modified stem cells in an infraction model; an improved functional recovery was obvious when compared with the transplantation of unmodified stem cells [12]. EPCs in healthy individuals may be a biologic marker for vascular function. Moreover, low levels of circulating EPCs may predict early atherosclerosis, occurrence of cardiovascular disorders, death from cardiovascular disorders [13] and prognosis after ischemic stroke [14]. The previous finding indicates that EPCs play an important part in the pathogenesis of atherosclerotic disease and assessment of EPCs may improve risk of cardiovascular disorders. This study aimed to prove that human EPCs can differentiate into cardiac myocytes after intramyocadial transplantation into canine with AMI. Phenotypic and functional biomarkers were assessed to prove myocardial differentiation.

Methodology

EPCs isolation from human umbilical cord blood

Five samples of human cord blood were enrolled in our after taken informed consents from women during caesarean sections labor. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. The blood mononuclear cell fraction (MNCs) was isolated from the buffy coats through density-gradient centrifugation with 20 ml Ficoll-Paque (Gibco-Invitrogen, Grand Island, NY). Centrifugation was for 35 min at 400*g*. The interphase layer of MNC was carefully aspirated and washed in PBS containing 2 mM EDTA and further centrifuged for 10 min at 200*g*°. The cell pellet was resuspended in 300 μl buffer and cultured for further cells propagation.

EPCs culture, propagation, labeling and counting based assay

EPCs were identified in culture by formation of a Colony Forming Unit (CFU) [5]. CFUs were formed after MNCs culturing for 7 days. For the EPCs counting assay, 5×10^6 MNCs were cultured onto fibronectin coated 96-well plates in M199 medium supplemented with 20% fetal calf serum (FCS), 0.1% human vascular endothelial growth factor-1 (VEGF-1) and 0.1% insulin-like growth factor (IGF-1) at 37 °C for 48 h. After seven days, EPCs were stained and further labeled with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocya-

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