The Role of Endothelial Progenitor Cells in Coronary Artery Disease: Basic Molecular Mechanisms and Its Clinical Potentials

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Received date: Apr 5, 2021; Revised date: May 27, 2021; Accepted date: May 27, 2021

Abstract

BACKGROUND: Coronary artery disease (CAD) remains as the world number one cause of morbidity and mortality. Endothelial progenitor cells (EPCs) are known to be involved in vascular biology. Current review briefly summarizes the basics of EPCs and its clinical use in CAD.

CONTENT: EPCs were firstly isolated in 1997 and involved in neovascularization. Further evidence defined EPCs into two distinguishable groups, namely: myeloid angiogenic cells (MACs) and endothelial colony forming cells (ECFCs). Common cardiovascular drugs, statin, angiotensin-converting enzyme (ACE) inhibitor, and their combinations, showed beneficial effects on EPCs. Likewise, the incorporation of EPCs upon CAD intervention management had been recently studied. Intramyocardial EPCs implementation and anti-CD34 antibody-coated stents could provide a promising option for refractory symptoms in CAD.

SUMMARY: Association between EPCs and CAD is very dynamic and complex. EPCs could serve as both therapeutic target and agent in CAD patients. Subsequently, a universal definition of EPCs is needed for greater research in the future.

KEYWORDS: atherosclerosis, coronary artery disease, endothelial progenitor cells, neovascularization

Introduction

In the past few decades, coronary artery disease (CAD) ranked as a leading cause of mortality, morbidity, and human suffering in both developed and developing countries. CAD is estimated to affect 126 million individuals and responsible for 9 million deaths globally. (1) Chronic endothelial dysfunction is widely known to develop into atherosclerosis and CAD, therefore the discovery of endothelial progenitor cells (EPCs) provides a promising future for CAD therapy. (2,3)

EPCs are generally described as monocyctic progenitor cells which initiate angiogenesis. Interestingly, EPCs behavior is shown to be associated with CAD pathophysiology, risk factors, and some cardiovascular drugs. Moreover, recent development and implementation of EPCs in CAD management endorse its clinical potency. Current review will summarize the basics of EPCs in CAD and its clinical potency.

Basic of EPCs

EPCs are generally interpreted as monocyctic progenitors with the ability to differentiate into endothelial cells and contribute to new blood vessel development. (3) The first discovery of EPCs is believed in 1932 after capillary-like
formations encountered in the culture of leukocytes. A forward to 1997, the first EPCs were isolated from peripheral human blood. The research beautifully demonstrated that cells which express cluster of differentiation 34 (CD34+) and vascular endothelial growth factor receptor-2 (VEGFR-2+) are involved in new blood vessel formation in mouse and rabbit model.

Many nomenclatures had been used to classify the type of EPCs. Most of them used hematopoietic and non-hematopoietic (early- and late-outgrowth (3), or circulating angiogenic cells and outgrowth endothelial cells (6). A novel nomenclature for this ambiguity had been proposed as myeloid angiogenic cells (MACs) and endothelial colony forming cells (ECFCs). MACs substitute terms of hematopoietic, early-outgrowth, and circulating angiogenic cells, whereas ECFCs for non-hematopoietic, late-outgrowth, and outgrowth endothelial cells.

Despite many nomenclatures, the different characteristics between those two are evident. MACs shape like a spindle and appear earlier in culture (less than 1 week) with a relatively short lifespan around 3-4 weeks, while ECFCs form cobblestone-like cells, appear around 2-4 weeks in culture with a longer lifespan duration (around 12 weeks). Fundamentally, both MACs and ECFCs have angiogenic potential. However, MACs are incapable to differentiate into endothelial cells and indirectly involved during angiogenesis by secreting interleukin 8 (IL-8) and vascular endothelial growth factor (VEGF) in a paracrine fashion. ECFCs, on the other hand, have the capability to differentiate into endothelial cells and directly involved in angiogenesis by forming vascular tubes in vitro or in vivo.

Identification of MACs and ECFCs merely based on surface markers is challenging. Many evidences showed slight different surface markers regarding those two. A study exploring surface markers among those EPCs showed that CD45 and CD133 were highly expressed in MACs, while VEGFR2, CD31, CD34, and vascular endothelial (VE)-cadherin were highly expressed in ECFCs. Another study showed MACs highly expressed CD45 and CD145 which can be a key surface marker for distinguishing MACs and ECFCs. It seems defining EPCs based on the surface antigen is quite burdensome since surface markers profile of EPCs may change over time during mobilization and maturation processes. Previous study also gracefully showed that surface markers were changing overtime among MACs and ECFCs.

In principle, the mechanisms of EPCs in neovascularization involve mobilization, homing, and differentiation into endothelial cells. During ischemia or vascular injury, VEGF and stromal cell-derived factor 1 (SDF-1) are upregulated and released in circulation. These substances activate endothelial nitric oxide synthase (eNOS) to produce nitric oxide (NO), followed by activation of matrix metalloproteinase-9 (MMP9). This MMP then cleavages kit ligand from membrane-bound (mKitL) into soluble kit ligand (sKitL) which releases niche EPCs resided in the bone marrow to circulation through c-Kit binding. There are also different recorded substances that facilitate EPCs mobilization, such as granulocyte (macrophage) colony-stimulating factor (GM-CSF), IL-8, C-X-C chemokine ligand 2 (CXCL2), erythropoietin, inflammatory cytokines, or drug (statins).

After being released into circulation, EPCs target and migrate to their respective location or “homing”. The EPCs homing mechanism consists of multistep sequences such as chemoattraction, adhesion, and transmigration. SDF-1 is considered as the most potent chemoattractant which is initially released by aggregated platelets and maintained by smooth muscle cells for days to weeks. Released SDF-1 forms a concentration gradient that navigates EPCs to a specific site through CXC receptor (CXCR4) on EPCs. During this process, SDF-1 also stimulates EPCs to express P-selectin glycoprotein ligand-1 (PSGL-1) and bind with the P-selectin on platelets.

Along with PSGL-1, the adjuvant structure also fortifies EPCs adhesion. MACs express β1- and β2-integrins, while ECFCs do not express β2-integrins. E-selectin may be related to these cells. These structures also mediate cell to cell interaction and EPCs transmigration to the respective location. In addition, other types of integrin, αvβ3- and αvβ5-integrin, appear to be involved in reendothelialization of denuded artery. The final step of homing is tissue invasion which still under investigation, yet it is hypothesized that cathepsins or MMP may be associated with this process.

The EPCs then play their role, which MACs indirectly aid angiogenesis by paracrine mechanism, while ECFCs are directly involved by differentiating into endothelial cells. The role of VEGF and NO are crucial for the differentiation process, yet the process is largely unknown. It is worth mentioning, there are still many unsolved questions regarding EPCs and its mechanism in vascular biology, thus further research of EPCs is necessary.
EPCs in CAD and Its Related Risk Factors

Despite limited information regarding EPCs, their involvement in vascular biology is evident. EPCs in CAD patients are not merely reduced in number, yet also become dysfunctional. An early study demonstrated that EPCs were 40% lower compared to healthy control. Moreover, impairment of its migratory response was also associated with CAD risk factors.(19) Number of EPCs are also related with CAD vessel involvement in which for every increase of 10 colony forming units (CFUs), multivessel CAD lowered by 20%.(20) This phenomenon may be caused by the inhibitory effect of oxidized LDL (oxLDL) on eNOS, thus compromising EPCs adhesive, migratory, tube formation, and survival in a dose-dependent fashion.(21)

Another study revealed a significant aspect of C-X-C chemokine receptor type 7 (CXCR-7) in EPCs function. Impaired ECFCs function of CAD patients appeared to be related with down-regulated CXCR7.(22) In fact, CXCR7 gene transfer to these cells significantly enhances cell adhesion and angiogenesis capacity through extracellular signal-regulated kinase (ERK) phosphorylation and vascular endothelial growth factor A (VEGFA) expression. Hence, the CXCR7/phosphorylated-ERK signaling pathway seems crucially implicated EPCs function in CAD patients. Attractively, a recent study demonstrated non-pharmacological intervention, namely shear stress, actually improves CXCR7 regulation together with EPCs proliferation, adhesion, and migration abilities.(23) However, the clear mechanism of how shear stress affects CXCR7 is yet to be elucidated.

During ischemic conditions, an acidic environment is developed. Low pH has inconclusive effects on EPCs. One study suggested an acidic environment inhibits EPCs function (24), while other evidence showed acidic preconditioning for EPCs is greatly increased survival and angiogenic ability under ischemic conditions (25). Despite these contrary results, the latest study prevailed role of G-protein-coupled receptor 4 (GPR4) as pH sensors and necessary for EPCs acid conditioning in CAD patients.(26) During the acidic condition, GPR4 is activated and induced signaling pathway involving by phosphorylating signal transducer and activator of transcription 3 (STAT3) and subsequent VEGFA expression, which boosts angiogenesis. Unfortunately, GPR4 expression is down-regulated in CAD patients, compromising EPCs function.

One interesting fact is that during acute conditions such as acute myocardial infarction (AMI), the number of EPCs increases. One systematic review demonstrated an increased number of EPCs (CD34+ or CFU-ECs) in patients with AMI and unstable angina compared to stable angina and healthy control.(27) One study had beautifully demonstrated EPCs traffic in AMI patients. CD34+ cells were increased to 5.8-fold in AMI patients within median duration of 195 minutes. This rapid mobilization peaked early after onset, decreased after 7 days, and normalized within 2 months. Higher plasma levels of SDF-1 and VEGF were also recorded, confirming their role as EPCs mobilizers. Additionally, previous evidence in AMI animal model exhibited EPCs cardioprotective feature through VEGF/VEGFR-2/p-Akt cascade, hence ameliorating eNOS function.(28)

Influence of cardiovascular risk factors on EPCs is still controversial. One systematic review consists of small study samples demonstrated that smoking, hypertension, diabetes mellitus type-1 or type-2, dyslipidemia, and aging were related to low number of EPCs.(27) Likewise, a study in metabolic syndrome population without diabetes and cardiovascular diseases revealed a significant reduction of EPCs number and function.(29) Negative effect of cardiovascular risk factors toward EPCs generally speculated by disruption of NO production. Diabetes mellitus and dyslipidemia lowers eNOS alteration via phosphatidylinositol-3 (PI-3) kinase/protein kinase B (PKB)/Akt/eNOS/nitric oxide signaling cascade.(30) Smoking induces oxidative stress and disrupts EPCs function possibly by methylation process and eNOS reduction.(31) Hypertension and aging are associated with decreased NO synthesis and increased oxidative species. (32) Besides, low growth hormone and insulin growth factor-1 during aging is also a possible mechanism in EPCs impairment.(33) Contrary to these findings, one study with 571 participants showed that there was a weak association between the number of EPCs and certain cardiovascular risk factors, yet a strong positive association with Framingham score which speculated as a protective mechanism available.(34) Further researches are needed to elucidate this difference.

EPCs as Therapeutic Targets of CAD Management

Studies had demonstrated that reduced EPCs is related to CAD severity, such as vessel involvement, disease progression, and SYNTAX score.(20,35,36) This reduced
EPCs may dampen vascular repair function, disrupting vascular injury-repair biology.(37) Therefore, EPCs could be considered as a promising therapeutic target in CAD management.

Marvelously, some cardiovascular drugs are proved to improved EPCs number and functions. Statins, a hydroxymethylglutaryl-coenzyme A (HMG-CoA) inhibitors, are well-documented for its pleiotropic effects in improving EPCs number, migration, and proliferation in CAD patients.(38-41) An in vivo study also demonstrated EPCs differentiation on statin treatment. This effect may be explained by PI-3 kinase/Akt cascade regulation by statins since inhibition of this cascade diminished EPCs improvement.(42)

Clinically speaking, high-intensity statins demonstrated more favorable effects in EPCs. Previous studies demonstrated that atorvastatin and rosuvastatin significantly improved EPCs migration compared to simvastatin.(40,43) On top of that, atorvastatin also demonstrated the highest enhancement in EPCs proliferation.(41) This evidence endorsed study in human which using pre-treatment atorvastatin before performing percutaneous coronary intervention (PCI). Administering 80 mg atorvastatin for 3 days before PCI in naïve CAD patient exhibited a 3.5 fold-increase in EPCs and persisted for 24-hour.(44)

A later study, HIPOCRATES Study, compared EPCs in patients receiving high- and low-dose statin therapy before PCI.(45) The high dose group received the first dose of 80 mg atorvastatin 18-24 hours before, the second dose of 40 mg 2-4 hours before, and a long-term dose of 20 mg after PCI procedure, while the low dose group only received 20 mg of simvastatin. The high dose group showed significant higher CFUs levels before PCI yet no difference after 24-hour post PCI compared to the low dose group. It is hypothesized that high levels of CFUs EPCs due to high dose statin provides a protective endothelial mechanism and reached the plateau phase, hence less prominent EPCs surge was observed.

Besides statins, ACE inhibitors are also known to have a pleiotropic effect on EPCs. This condition is probably mediated through bradykinin B2-receptor cascade and upregulation of eNOS.(46) CAD patients who received 5 mg of ramipril each day for 4 weeks manifested an increase of 1.5-fold and 2.5-fold EPCs by week-1 and -4, respectively.(47) Furthermore, its proliferation, migration, adhesion, and tube-forming capacity were also markedly improved along with increased NO and decreased systolic blood pressure.

Another study compared enalapril 20 mg with zofenopril 30 mg in newly diagnosed hypertensive patients for 5 years follow-up.(48) This study demonstrated similar increase of EPCs between groups and marked inverse correlation between EPCs number and carotid intima media thickness. A recent study tested various types of ACE inhibitors and observed interesting findings. Captopril, ramipril, and lisinopril were proved to enhance EPCs migration in a dose-dependent fashion.(49) At the given dose, 1 mM and 10 mM, captopril outperformed other ACE inhibitors. However, for 100 mM dose, lisinopril took the lead.

The latest evidence also endorsed secretomes influence on EPCs. In a recent study conducted by the authors, administration of human umbilical cord blood mesenchymal stem cells derived secretome was found to significantly improve the function (proliferation and migration) of EPC derived from CAD patients.(50) This effect was synergistic with the concomitant administration of statins and also ACE inhibitors. Despite the lack of exact mechanisms and compositions of secretomes that are still under-investigated, these findings could become the basis for the use of secretomes as a new modality for CAD therapy.

### EPCs in CAD Therapeutics

In stable CAD, the number and function of circulating CD34(+) CD133(+) progenitor cells decreased with age, whereas those mobilized and circulating in AMI did not. (51) Although vast advancements in CAD treatment, in some cases, symptoms may persist even with optimal intervention. This condition is referred as refractory angina (RA) or ischemic cardiomyopathy (ICM).(52,53) Growing evidence supports intramyocardial autologous CD34+ cells implantation for RA.

Previously, two trials had been conducted to evaluate CD34+ cells intramyocardial therapy. The initial study, ACT34-CMI Study, enrolled 24 participants and evaluated 3 different doses of CD34+ cells injection: \(5 \times 10^4\), \(1 \times 10^5\), and \(5 \times 10^4\) cells/kg.(54) Marked reduction in angina frequency, severity based on Canadian Cardiovascular Society (CCS) class, and nitroglycerine usage were documented. Additionally, slight improvements were also observed in exercise tolerance and perfusion imaging by single-photon emission computerized tomography (SPECT). Incidence of serious adverse events was also distributed among participants. Another randomized controlled trial
revealed that coronary artery bypass graft (CABG) using trans-epicardial and trans-septal autologous CD133(+) bone marrow cells implantation improved left-ventricular function in low EF coronary artery disease patients. CD133(+) progenitor cells improve cardiac function and repair the myocardium by stimulating neovascularization and angiogenesis.(55)

Later, phase II ACT34-CMI Study was published in 2011 enrolling a total of 167 “no-option” RA patients. (56) This study evaluated 2 doses, 1×10⁶ and 5×10⁶ cells/kg, for 12 months. Interestingly, low-dose CD34+ therapy outperformed other groups. Weekly angina frequency, exercise tolerance, and SPECT imaging results were significantly improved compared to control and high-dose group during 6 months and 12 months follow-up. Improvement was also observed in CCS class reduction and nitroglycerine usage. Major adverse cardiac events (MACEs) were not different among groups, yet no deaths were observed in the treated group compared to control group (5.4%).

Further observation in 24 months also revealed a significant reduction in angina frequency in both low- and high-dose compared to control group.(57) MACEs rate was significantly reduced in treated groups (21.8% for low- and 16.2% for high-dose) compared to control (33.9%). Specifically, mortality rate was also lower in the treated group (1.8% for low- and 3.6% for high dose) compared to control (12.5%). Recently, a trial design for phase III had been planned.(58)

A different trial, RENEW Trial, focused on CD34+ intramyocardial injection towards total exercise time (TET).(59) This trial compared 3 following groups: the treatment group received intramyocardial autologous CD34+ cells dosed from 1×10⁶ to 1×10⁷ cells/kg, G-CSF stem cell mobilization, apheresis; active control received the same regiment with treatment group except for CD34+ injection; and standard control without any intervention. Unfortunately, the trial was terminated early, hence the results should cautiously be interpreted. Overall, CD34+ showed an improvement in TET, yet was not statistically significant compared to active control. Angina frequency was markedly reduced in the treatment and active control group, yet the relative risk for angina was only significant in 6 months follow-up. (RR=0.58, p=0.02).

Besides intramyocardial implantation, EPCs had been combined with PCI procedure. During balloon or stent deployment, endothelial lining of the coronary artery is disrupted, leading to neointimal hyperplasia and restenosis. Therefore, coating stents with anti-CD34 antibodies to capture CD34+ cells seems to be rational since these cells are involved in vascular healing.(60)

The first human study using this stent was HEALING First in Man (FIM) study. HEALING FIM study was a nonrandomized, prospective, single-center study with 16 de novo CAD patients.(61) At 6 months follow-up, most angiographic morphology remains similar to post-PCI, mean late luminal loss was 0.63±0.52 mm and 27.2±20.9% in-stent restenosis. The MACEs and cerebrovascular events rate were 6.3%, despite only 1-month dual antiplatelet therapy. This result initiated later studies, such as HEALING II, e-HEALING, and HEALING IIb.(62)

Another trial, the TRI-stent adjudication study (TRIAS), was a randomized, prospective, single-center study composed of 193 participants.(62) The study compared EPCs-capture stents (ECS) to paclitaxel-eluting stents (PES) in de novo lesions with a high risk of restenosis. At 6 and 12 months of follow-up, PES outperformed ECS with 0.55±0.61 mm and 1.14±0.64 mm in-stent late loss, respectively. Further, PES also demonstrated lower target-vessel failure, even not statistically significant (10.5% vs. 17.3%, p=0.172). However, the result may be underpowered due to study design transformation into an international multicenter study. The TRIAS trial was divided into two classes which compared ECS with drug-eluting stent (DES) in TRIAS-HR and bare-metal stent (BMS) in TRIAS-LR toward MACEs and clinical target lesion revascularization.(63) Surprisingly, ECS did not provide any difference to DES at 1 and 2 years follow-up.(64,65) Five years follow up of TRIAS-LR also exhibited similar results.(66)

Despite the unsatisfying performance of ECS to DES in terms of target lesion failure, a combination of EPCs capturing technology along with Sirolimus elution had been introduced as COMBO dual-therapy stent. This combination considers reendothelialization aspects of EPCs as well as restenosis prevention of Sirolimus. Recent trials (REMEDEE, REMEDEE-OCT, and Japan-USA HARMONEE) combined DES with anti-CD34 antibody coating and compared it toward DES. Interestingly, the combined stent showed a non-inferiority result toward DES.67 However, as several limitations occurred in those trials, (i.e., relative less complex lesion, <5% acute coronary syndromes, and <12% multivessel diseases), further studies with better designs are required to confirm those non-inferiority results could be applied in more complex patients and coronary artery lesion.(68)
The Indonesian Biomedical Journal, Vol.13, No.2, June 2021, p.106-220

Conclusion

Association between EPCs and CAD is very dynamic and complex. EPCs could be served as both therapeutic target and agent in CAD patients. Despite many trials performed, a universal definition of EPCs is needed to boost further research in the future.

Authors Contribution

YHO and SAH were involved in concepcting and planning the research. YHO, SAH and KEV collect the information/literature. YHO, SAH and KEV drafted the manuscript. YHO, SAH and KEV took parts in giving critical revision of the manuscript.

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