Endothelial progenitor cells as therapeutic vectors in cardiovascular disorders: From experimental models to human trials

Jeremy Ben-Shoshan, Jacob George

Department of Cardiology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel
Tel Aviv University, Sackler School of Medicine, Tel Aviv, Israel

Abstract

Cell-based therapy approaches for the restoration of blood flow in ischemic organs has recently received growing interest. A considerable number of reports have documented the presence of circulating, bone marrow-derived endothelial progenitor cells (EPC) in adult peripheral blood. These putative cells are thought to participate in postnatal growth of new blood vessels. Mounting evidence from animal studies point to potential therapeutic applications of EPCs in the treatment of a wide range of cardiovascular (CV) disorders, while preliminary results from the pilot clinical trials still remain equivocal. Here, we review the experimental data that has accumulated so far from animal and clinical studies regarding the potential importance of EPCs. In addition, we discuss the potential hurdles as well as future options of EPC-based therapy.

Keywords: Endothelial progenitor cells; Bone marrow; Peripheral blood; Mobilization; Cell-based therapy; Cardiovascular diseases

Abbreviations:
AMI, acute myocardial infarction; BM, bone marrow; BMC, bone marrow cells; BM-MNC, bone marrow mononuclear cells; CABB, coronary artery bypass grafting; CCSAS, Canadian Cardiac Association Angina Score; CFR, coronary flow reserve; CHF, chronic ischemic heart failure; CV, cardiovascular; DB, double blinded; EF, ejection fraction; eNOS, endothelial nitric oxide synthase; EPCs, endothelial progenitor cells; Epo, erythropoietin; G-CSF, granulocyte-colony stimulating factor; IS, infarct size; LV, left ventricle; LVEF, left ventricular ejection fraction; LVES, left ventricular end systolic volume; LVWM, left ventricular wall motion; MI, myocardial infarction; MMP, matrix metalloproteinases; MP, myocardial perfusion; NR, nonrandomized; PB, peripheral blood; PB-EPC, peripheral blood endothelial progenitor cells; PB-MNC, peripheral blood mononuclear cells; PCI, percutaneous coronary intervention; PVD, peripheral vascular diseases; R, randomized; RC, randomized controlled; RD, reversible defect; SDF-1, stromal cell derived-factor-1; SV, stroke volume; UC, uncontrolled; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.
1. Introduction

Until a decade ago, the creation of new blood vessels, termed vasculogenesis, was thought to occur only during embryonic development. Postnatal revascularization of tissues was referred to as a distinct mechanism, angiogenesis, namely, the process of branching and elongation of existing vessels by migration and proliferation of resident mature endothelial cells (Risau, 1997). This dogma was revolutionized by Asahara et al. (1997), who identified for the first time, circulating endothelial progenitor cells (EPC) in adult peripheral blood (PB). In this study, EPCs were defined as bone marrow (BM)-derived cells, capable of trafficking toward ischemic sites and differentiating into mature endothelial cell. Since then, the mechanisms by which EPCs participate in capillary formation are being thoroughly investigated using a variety of animal models. Additionally, different techniques, such as induced mobilization and genetic modulations, have been considered in order to improve the vasculogenic properties of EPCs.

Consistent with the widely accepted notion that adult BM is a source of various stem/progenitor cells and based on experimental studies, a growing number of clinical trials are testing the feasibility and safety of cellular therapy for the treatment of ischemic cardiovascular (CV) diseases. One major objective of those trials, along with direct tissue regeneration, is to restore the perfusion of ischemic organs, by enhancing the local and systemic levels of EPCs. The key issues in this context concern the most appropriate cell population to use and the best means of delivery in order to generate effective strategies for future therapeutic neovascularization.

2. Endothelial progenitor cell phenotype

As the principal cellular components of the embryonic vascular network, angioblasts and hematopoietic stem cells share several antigenic determinants, including CD34, CD117 (c-Kit), vascular endothelial (VE) growth factor (VEGF) receptor-2 (VEGFR2) and angiopoietin-1 receptor (Tie2). Consequently, they have been considered to arise from a common putative precursor, termed hemangioblast (Peichev et al., 2000). Recently, accumulating evidence suggests that persistence of the hemangioblast into adulthood contributes to the maintenance and repair of both hematopoietic and endothelial lineages (Pelosi et al., 2002). Additional evidence for the common origin of those 2 lineages arises from several studies showing high expression of CD14 myelo-monocytic marker on circulating EPCs (Harraz et al., 2001).

The precise point of diversion of the hemangioblast to angioblast and subsequently to EPC has not yet been fully determined. Nevertheless, a distinction is made between CD34+/CD133+/VEGFR2+ early EPCs and late EPCs which lose CD133 and begin to express endothelial lineage cell markers, including CD31, von-Willebrand factor (vWF), VE cadherin, and endothelial nitric oxide synthase (eNOS) (Asahara et al., 1997). Interestingly, a parallel division between early and late circulating EPCs was recently described by Hur et al. (2004) who demonstrated 2 phenotypically discrete types of EPCs in vitro with differential proliferative capacity, but yet with comparable in vivo vasculogenic efficacy.

3. Endothelial progenitor cell culture

The main sources for the isolation of EPCs are the PB and the BM. Following separation of the mononuclear cells (MNC) fraction, EPCs can be enriched by direct cell sorting (i.e., flow cytometry or magnetic beads), using either a single or combination of antibodies directed against specific markers (Fig. 1). Indeed, different researchers employ distinct marker-based characterization in order to assess and isolate EPCs (Asahara et al., 1997; Shi et al., 1998; Peichev et al., 2000). In a recent comparative analysis of the different methods employed for EPC assessment, we concluded that CD34+/VEGFR2+ phenotype is apparently the more appropriate for the definition of circulating EPCs (George et al., 2006). However, the number and culture quality of EPCs achieved by sorting methods is often limited. Therefore, whole MNCs culture is frequently employed for efficient expansion of EPCs. MNCs are plated on fibronectin-coated dishes and maintained in endothelial selective medium supplemented by proangiogenic cytokines (i.e VEGF, bFGF, EGF). After 7–10 days in culture, colonies of spindle shape, endothelial-like, adherent cells are formed. At this stage, a large majority of the cells show expression of endothelial cell markers such as VEGFR2, vWF, and CD31. In addition, the cells obtain typical endothelial properties such as endocytosis of acetylated low-density lipoprotein and binding of Ulex europaeus agglutinin-1. Eventually, in order to assess the function of EPCs, they are usually tested in vitro for their ability to adhere and form capillary-like tubes when seeded on matrix components, as well as for their migration capacity toward angiogenic stimuli.

4. Mobilization of endothelial progenitor cells

The local cellular response, evoked in ischemic areas, involves the release of various growth factors and cytokines that promote the recruitment of EPCs (Takahashi et al., 1999). This response is principally regulated by hypoxia-inducible transcription factors (HIFs), activated under low oxygen levels, which drive the expression of multiple proangiogenic factors that stimulate tissue revascularization (Semenza, 2003). The hypoxic gradient-mediated trafficking of EPCs has been shown to occur through the HIF-1-mediated induction of stromal cell-derived factor-1 (SDF-1)
expression in hypoxic endothelial cells (Ceradini et al., 2004). Binding of SDF-1 to CXCR4 receptor on circulating progenitor cells enhance their migration, adhesion, and homing to ischemic tissues. An additional major HIF-1-regulated mediator of EPC mobilization is VEGF. Indeed, in patients after acute myocardial infarction (AMI; Shintani et al., 2001) or vascular trauma (Gill et al., 2001), the number of circulating EPCs considerably increases, in direct correlation with serum VEGF levels. Moreover, VEGF administration has been shown to effectively increase EPC levels in the peripheral blood, both in animal models (Asahara et al., 1999) and clinical pilot trials (Kalka et al., 2000a). Recently, VEGF-induced migration of EPCs was demonstrated to be mediated both by VEGFR1 (flt-1) and VEGFR2 (flk-1), as opposed to the mechanism of mature endothelial cell migration that is principally induced via VEGFR2 (Li et al., 2006). Erythropoietin (Epo), also controlled by HIF-1, has been suggested as an important physiological determinant of EPC mobilization. In fact, prolonged Epo treatment in chronic heart failure (George et al., 2005b; Schwartzenberg et al., 2006), coronary artery disease (Heeschen et al., 2004), and renal anemia (Bahlmann et al., 2003) patients was found associates with increased number and improved functional properties of circulating EPCs.

Cytokines promoting granulocyte proliferation and mobilization also affect BM-derived progenitor recruitment. Both granulocyte-colony stimulating factor (G-CSF; Takahashi et al., 1999; Kocher et al., 2001) and granulocyte macrophage-colony stimulating factor (GM-CSF; Natori et al., 2002) have been shown to induce EPC mobilization. In addition, genetic cell-labeling techniques have obviously demonstrated incorporation of G-CSF and GM-CSF-mobilized progenitors into the growing vasculature of ischemic tissues. Recently, G-CSF was found to stimulate VEGF release from neutrophils, facilitating an “angiogenic environment” to further promote local EPC recruitment (Ohki et al., 2005). Interestingly, combined therapy of G-CSF administration with sustained delivery of bFGF further increased capillary density following induction of hind-limb ischemia in mice, as compared with either strategy alone (Jeon et al., 2006). Combined treatment of G-CSF/GM-CSF (Bruno et al., 2006) and GM-CSF/SDF-1 (Atluri et al., 2006) were also investigated and found to increase the levels of cell populations with proangiogenic properties, which eventually contribute to vessel formation.

Estrogen was also found to increase circulating EPC number and mitogenic activity, leading to accelerated reendothelialization and decreased neointimal formation in injured vessels (Strehlow et al., 2003; Iwakura et al., 2003). These effects of estrogen were found to be eNOS-dependent, consistent with previous findings that point at the importance of eNOS expression in the BM-EPC release regulation (Aicher et al., 2003). Moreover, the antiapoptotic effects of estrogen on EPCs were associated with an increased telomerase activity and enhanced VEGF secretion (Imanishi et al., 2005a).

Several pharmacological agents were found to increase EPC mobilization. During the past few years, the effects of cholesterol-

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**Fig. 1.** EPC-based strategy for cellular therapy. In the body, BM-derived EPCs are mobilized to the PB circulate toward ischemic tissue and participate in neovascularization. For ex-vivo expansion, MNCs can be isolated from either BM or PB by density gradient centrifugation. Specific progenitor cell fractions can be directly sorted from these sources by magnetic beads or flow cytometry using surface markers, such as CD34, CD133, and VEGFR2. The isolated cells are then cultured in endothelial selective conditions for 4–7 days. Genetic modulations can be produced during the culture period to improve EPC angiogenic properties. EPCs can be then retransplanted to induce ischemic tissue revascularization.

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Please cite this article as: Ben-Shoshan, J., & George, J. Endothelial progenitor cells as therapeutic vectors in cardiovascular disorders: From experimental models to human trials. *Pharmacol Ther* (2007), doi:10.1016/j.pharmthera.2007.03.012
lowering drugs HMG-CoA reductase inhibitors (statins) have been attributed to multiple “nonmetabolic” biological effects, including antiinflammatory and antithrombotic properties (Packard, 1998). Likewise, enhanced coronary blood flow and reduced myocardial ischemia were observed in statin treated-patients with coronary artery disease. Lately, simvastatin has been shown to markedly induce BM-EPC mobilization and integration into developing vessels, leading to improved left ventricular (LV) function after MI in mice (Llevadot et al., 2001). Moreover, atorvastatin treatment has been found to rapidly increase the differentiation rate and number of circulating EPCs in patients with stable coronary artery disease compared with patients treated with VEGF (Vasa et al., 2001). In a study conducted on type-2 diabetic patients by Pistrosch et al. (2005), the PPARγ-agonist rosiglitazone was observed to enhance EPC numbers and their migratory capacity. Similarly, in response to ischemic stress, mice treated with enalapril, an angiotensin-converting enzyme (ACE) inhibitor, exhibited an increase in circulating EPC numbers with increased angiogenic capacities (Wang et al., 2006). Interestingly, enalapril-treated animals showed higher SDF-1 concentration gradient between the BM and the PB compared with controls.

Several studies had focused on the effect of physical training on circulating EPC numbers. Different terms of endurance training in healthy subjects, as well as in patients with CV risk factors or coronary artery disease, led to a considerable increase in circulating EPCs (Laufs et al., 2004). In humans, exercise-induced increase in EPC numbers was correlated with VEGF plasma levels, nitric oxide synthesis, and vascular function (Steiner et al., 2005). Murine angiogenesis models showed an increase in neoangiogenic capacity and inhibition of neointimal formation after carotid injury, following exercise activity (Laufs et al., 2004).

Different factors have been shown to decrease circulating EPC levels, suggesting potential beneficial effects in antiangiogenic therapy, such as those implicated for cancer treatment. Angiotatin, an antiangiogenic cleavage product of plasminogen, was shown to reduce the proliferation and colony-forming ability of EPCs in humans (Ito et al., 1999). Likewise, tumor-bearing animals treated with endostatin, an additional proteolytic cleavage product, exhibited a reduced tumor-associated increase in EPCs, as well as inhibited tumor growth (Capillo et al., 2003).

The exact molecular mechanisms by which progenitor cells are stimulated and released from the BM environment to the circulation remain poorly understood. Matrix metalloproteinases (MMP) have been suggested by Heissig et al. (2002) to play an essential role in this process by promoting the binding of Kit ligand to cKit+ progenitor cells, which will be subsequently mobilized to the circulation. Indeed, VEGF-induced EPC mobilization was significantly reduced in MMP9−/− mice or following administration of a MMP inhibitor. However, further studies are required to further confirm and elucidate those mechanisms.

5. Endothelial progenitor cells: diagnostic markers of vascular dysfunction

Endothelial dysfunction potentially predispose to several common CV disorders (Lusis, 2000). Pathological conditions such as hyperlipidemia, hypertension, diabetes, and inflammation expose the vessel wall to continuous injury, leading to disruption of endothelial integrity. Consequently, the vessel wall is faced with increased vascular tone, leukocyte, and platelet accumulation, thrombus formation, oxidative stress, smooth muscle proliferation, and eventually atherosclerotic lesion formation.

5.1. Ischemic cardiac diseases

Assessment of EPC numbers and functional properties have led to the notion that these cells play a key role in maintenance of vascular hemostasis in patients with ischemic coronary disorders. Human studies have shown a negative correlation between PB-EPC levels and the presence of CV risk factors, occurrence of CV events, and death from CV causes (Hill et al., 2003; Werner et al., 2005a, 2005b). Indeed, circulating EPC number was found to predict vascular reactivity impairment more accurate than conventional risks factors as defined by Framingham risk factor score. Likewise, EPCs isolated form patients with coronary artery disease or multiple CV risk factors have been associated with impaired proangiogenic function and higher in vitro-senescence rates, compared with controls (Rivard et al., 1999; Rauscher et al., 2003). These observations are consistent with the theory that continuous risk-factor-induced endothelial dysfunction may eventually lead to the exhaustion of a BM-derived progenitors that participate in vascular maintenance. This concept is supported by studies demonstrating an age-related decline in BM-EPCs, suggestive of an additional explanation of the age-associated CV morbidity and mortality (Hill et al., 2003).

Several studies have demonstrated an elevation in circulating EPC levels in conditions associated with tissue ischemia, suggesting an endogenous compensatory revascularization mechanism. As mentioned previously, after AMI, EPC levels increased, peaking on day 7, in correlation with VEGF plasma levels (Shintani et al., 2001). In a study conducted on heart failure patients by Valgimigli et al. (2004), mobilization of EPCs was found to occur in a biphasic manner, with elevation and depression in the early and advanced phases, respectively, mirroring TNFα plasma levels fluctuations. In patients with unstable angina pectoris, we have recently found elevated EPC numbers compared with subjects with stable angina and in correlation with systemic inflammation, tested by plasma C-reactive levels (George et al., 2004). Similarly, in patients with cardiac syndrome X, characterized by angina-like chest pain with normal coronary arteries, elevated numbers of EPCs were evident, but with impaired capillary formation capacity, pointing to the possibility of impaired endothelial function in these individuals (Shmilovich et al., 2006).

An additional field in which EPCs appear to be protective is vascular complications following invasive interventions, such as transplantation and percutaneous coronary intervention (PCI). For example, EPCs were shown to contribute to endothelial regeneration in murine autologous vein graft model (Xu et al., 2006) and human cardiac allografts (Simper et al., 2003). Moreover, among cardiac allograft recipients, lower levels of circulating EPCs were observed in these patients exhibiting...
allograft vasculopathy. In patients after implantation of intracor-
onary stents, intact endothelialization is a prerequisite to prevent
neo-intimal formation with subsequent in-stent restenosis. In this
case, we have recently found a negative correlation between the
diffuseness of in-stent restenosis and circulating EPC number and
functional properties (George et al., 2003). These findings
emphasize the potential contribution of EPCs in vascular
maintenance and regeneration.

5.2. Peripheral vascular diseases

Several studies have explored the role of EPCs in the patho-
physiology of different peripheral vascular diseases (PVD). The
number and proliferation rate of EPCs obtained from both type I
and type II diabetes patients exhibited a 2-fold decrease relative to
healthy subjects, a trend that inversely correlated with the levels of
hemoglobin A1C and glucose levels (Tepper et al., 2002; Loomans et al., 2004). Additionally, in diabetic patients,
migration, adhesion, and endothelial chemoattractiveness of EPCs
were significantly reduced. Diabetes-induced PVD was
found to be associated with a 47% reduction in EPC levels,
correlating with the ankle-brachial index (Fadini et al., 2005).
Different mechanisms have been suggested to explain these
findings, including up-regulated expression of thrombospondin-1
(Ti et al., 2006), down-regulated EPC expression of eNOS, and
inability of diabetic EPCs to respond to VEGF and SDF-1
chemoattractant activity (Segal et al., 2006).

Patients with chronic renal failure were also characterized by
a low number and hampered function of EPCs (Choi et al.,
2004), and EPC senescence was found to be accelerated in
patients with essential hypertension, potentially relating to
telomerase inactivation (Imanishi et al., 2005b). Collectively,
these findings may partially explain the increased CV risk in
these patients.

6. Progenitor cell-based therapeutic neovascularization

During the last decade, numerous reports have documented
the potential of autologous cell-based therapeutic strategies for
regeneration of ischemic organs. Considerable effort has been
made to show that enhancing the levels of circulating and
recruited EPCs will facilitate revascularization of ischemic
tissues.

6.1. Animal studies

In a swine MI model, transcendocardial catheter-based
administration of autologous PB-MNCs (Kawamoto et al.,
2003), PB-CD31+ cells (Kamihata et al., 2002), or BM-MNCs
(Fuchs et al., 2001) was found to improve capillary density,
collateral flow, and left ventricular ejection fraction (LVEF) 3–
6 weeks after implantation. Similar results were achieved
employing, intramyocardial injection of autologous BM-MNCs
(Kobayashi et al., 2000) or Lin−/c-Kit+ cells (Orlic et al., 2001) in
murine MI models. Intramyocardial (Kawamoto et al., 2001) or
systemic (Kocher et al., 2001; Kawamoto et al., 2003) injection of
human CD34+ cells in nude rats was also associated with in-
creased capillary density and LVEF, while infarct size and
cardiomyocyte apoptosis were significantly reduced. EPC
transfer benefits were also assessed for the treatment of PVD
using animal hind-limb ischemia models. In these studies, both
intramuscular injection of autologous BM-MNCs (Ikenaga et al.,
2001) or systemic injection of human PB-MNCs (Kalka et al.,
2000b; Iba et al., 2002; Iwaguro et al., 2002) were associated with
increased capillary formation, muscle perfusion, and exercise
capacity. Interestingly, EPCs were also found to contribute to the
infarct border and choroid plexus neovascularization after
cerebral ischemia, despite the complex structure and rare turn
over of the cerebral endothelium (Zhang et al., 2002). Notably,
the ability of progenitor cells to incorporate into sites of neovascular-
ization has been well demonstrated during these experiments
using various techniques of cell genetic labeling.

The efficacy of EPC-based therapeutic neovascularization
has been also explored in different nonischemic CV diseases.
Recently, we have shown protective effects of EPC in dilated
cardiomyopathy (DCM) following autoimmune myocarditis in
rats (Werner et al., 2005a, 2005b). In this model, transfer of
EPCs resulted in a functional improvement in cardiac performance
evident by higher fractional shortening as well as reduced
scar tissue and thickened ventricular walls. We have demonstra-
ated beneficial effects of EPC transfer in doxorubicin-induced
cardiomyopathy in rats (Hamed et al., 2006). Finally, trans-
plantation of human EPCs was found to reverse the development of
diabetic neuropathy in diabetic nude rats (Naruse et al., 2005).

6.2. Clinical trials

The encouraging results achieved in the experimental animal
experiments prompted several groups to conduct pioneering
clinical studies in order to test the feasibility and safety of EPC-
based approaches for the treatment of ischemic CV diseases
(Table 1). Most of the trials established so far were
nonrandomized and unblinded, but yielded preliminary results
that show this approach to be relatively safe and potentially
beneficial.

6.2.1. Acute myocardial infarction

The most consistent improvement in cardiac function attained
by means of cell-therapy has come from studies using autologous
BM-derived cells after AMI. One of the earliest small-scale
nonrandomized trials (Strauer et al., 2002) assessed intracoronary
injection of autologous BM-MNCs in 10 patients 7 days after
AMI. After 3 months, the investigators reported reduced infarct
size and improved myocardial motion compared with control
subjects treated with standard therapy. Following this study, the
TOPCARE-AMI (transplantation of progenitor cells and regen-
eration enhancement in AMI) trial (Schachinger et al., 2004)
evaluated the efficacy of intracoronary catheter-based adminis-
tration of either PB-MNCs or BM-MNCs in 105 patients 7 days after
AMI. After 3 months, the investigators reported reduced infarct
size and improved myocardial motion compared with control
subjects treated with standard therapy. Following this study, the
TOPCARE-AMI (transplantation of progenitor cells and regen-
eration enhancement in AMI) trial (Schachinger et al., 2004)
evaluated the efficacy of intracoronary catheter-based adminis-
tration of either PB-MNCs or BM-MNCs in a total of 59 patients
with reperfused AMI. Notably, after 3 days of ex vivo expansion,
more than 90% of the injected cells exhibited endothelial charac-
teristics. At 4 months of follow-up, quantitative LV angiography
revealed a significant increase in LVEF and a decrease in end
systolic volumes in both treated groups. After 12 months,
magnetic resonance imaging (MRI) reconfirmed the improvement in LVEF and showed a reduced infarct size, as well as absence of reactive hypertrophy, suggesting functional LV regeneration following cell therapy.

The first randomized controlled trial of progenitor cell therapy was the BOOST (bone marrow transfer to enhance STY-elevation infarct regeneration) trial conducted by Wollert et al. (2004) in which autologous BM-MNCs were injected in the infarcted related arteries of 30 post-MI patients 5 days after successful PCI. As determined by cardiac MRI after 6 months, global LVEF was improved in patients treated with progenitor cells. Transfer of BM cells was also associated with increased LV systolic function primarily in myocardial segments adjacent to the infarcted area. Interestingly, a subsequent study (Hofmann et al., 2005), using fluoro-deoxy-glucose and 3D photon emission tomography imaging, to monitor myocardial homing after cells delivery, estimated an engraftment of 14–39% of these transferred cells. Similar results were reported by Ruan et al. (2005) in the first, small, double-blind randomized, controlled study which has demonstrated LVEF improvement by echocardiography following intracoronary BMCs administration in 20 patients 6 months after MI.

Recently, a multi-center, double-blind, placebo-controlled, randomized study involving 204 patients at 17 medical centers has been completed in Germany and Switzerland (Schachinger et al., 2005). The REPAIR-AMI (reinfusion of enriched progenitor cells and infarct remodeling in AMI) trial evaluated the effects of intracoronary delivery of progenitor cells after MI and primary PCI. Patients were randomized to receive an infusion to infarct-related artery of autologous BM-MNCs group or placebo, 3–6 days after AMI. At 4 months, a significant increase in LVEF was observed by LV angiography in the cell-treated group compared with placebo. Interestingly, this relative increase was evident only in patients with a baseline of EF < 49% and, in addition, in patients treated a minimum of 5 days after MI. Even though the study was not powered to detect a difference in clinical end points, a trend toward a reduction in the composite of death, MI, or repeat revascularization was evident in the treatment group (21% vs. 30%).

6.2.2. Chronic ischemic heart failure

Several clinical trials have investigated the use of progenitor cell transfer for the in-patients with heart failure. Cell delivery methods varied between different studies. In a prospective, nonrandomized, open-label study (Perin et al., 2003) transendocardial catheter-based cell administration has been used to deliver BM-MNCs to 14 patients with severe chronic ischemic heart failure (CHF) and no option for standard revascularization therapies. After 2 months of follow-up, a significant decrease was evident in total reversible perfusion

<table>
<thead>
<tr>
<th>Group/study</th>
<th>End point year</th>
<th>Design</th>
<th>No. of treated patients</th>
<th>Cell type</th>
<th>Delivery route</th>
<th>Follow up (months)</th>
<th>Main outcomes</th>
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<tbody>
<tr>
<td>AMI</td>
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<tr>
<td>Strauer et al.</td>
<td>2002</td>
<td>NR</td>
<td>10</td>
<td>BM-MNCs</td>
<td>Intracoronary</td>
<td>3</td>
<td>↑ SV, LVESV, LVWM, MP ↓ IS</td>
</tr>
<tr>
<td>TOPCARE-AMI</td>
<td>2004</td>
<td>UC</td>
<td>30/29</td>
<td>BM-MNCs/PB-EPCs</td>
<td>Intracoronary</td>
<td>4,12</td>
<td>↑ LVEF, LVWM, CFR ↓ LVEF, LVWM, IS</td>
</tr>
<tr>
<td>BOOST</td>
<td>2004</td>
<td>RC</td>
<td>30</td>
<td>BM-MNCs</td>
<td>Intracoronary</td>
<td>6</td>
<td>↑ LVEF, MP, Restenosis ↓ LVEF, LVWM, CFR</td>
</tr>
<tr>
<td>MAGIC</td>
<td>2004</td>
<td>RC</td>
<td>10</td>
<td>G-CSF+PB-MNCs</td>
<td>Intracoronary</td>
<td>6</td>
<td>↑ LVEF, LVWM, WM ↓ LVEF, LVWM, CFR</td>
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<td>NR</td>
<td>20</td>
<td>BM-MNCs</td>
<td>Intracoronary</td>
<td>6</td>
<td>↑ LVEF, LVWM, CFR ↓ IS</td>
</tr>
<tr>
<td>Erbs et al.</td>
<td>2005</td>
<td>R, DB</td>
<td>13</td>
<td>PB-EPCs</td>
<td>Intracoronary</td>
<td>3</td>
<td>↑ LVEF, LVWM, CFR</td>
</tr>
<tr>
<td>Kuethe et al.</td>
<td>2004</td>
<td>UC</td>
<td>5</td>
<td>BM-MNCs</td>
<td>Intracoronary</td>
<td>3,12</td>
<td>No improvement</td>
</tr>
<tr>
<td>Ruan et al.</td>
<td>2005</td>
<td>R, DB</td>
<td>9</td>
<td>BM-MNCs</td>
<td>Intracoronary</td>
<td>6</td>
<td>↑ LVEF</td>
</tr>
<tr>
<td>ASTMI</td>
<td>2005</td>
<td>RC</td>
<td>50</td>
<td>BM-MNCs</td>
<td>Intracoronary</td>
<td>12</td>
<td>No improvement</td>
</tr>
<tr>
<td>REPAIR-AMI</td>
<td>2006</td>
<td>R, C, DB</td>
<td>102</td>
<td>BM-MNCs</td>
<td>Intracoronary</td>
<td>4</td>
<td>↑ LVEF, CRF</td>
</tr>
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<td>CHF</td>
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<tr>
<td>Hamano et al.</td>
<td>2001</td>
<td>UC</td>
<td>5</td>
<td>BMCs</td>
<td>Trans-epicardial</td>
<td>12</td>
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<td>2003</td>
<td>NR</td>
<td>14</td>
<td>BM-MNCs</td>
<td>Trans-endocardial</td>
<td>2,4</td>
<td>↑ LVEF</td>
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<td>Stamm et al.</td>
<td>2003</td>
<td>UC</td>
<td>6</td>
<td>CD133+ BM-EPCs</td>
<td>Intra-myocardial</td>
<td>3,9</td>
<td>↑ LVEF, MP</td>
</tr>
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<td>2003</td>
<td>UC</td>
<td>10</td>
<td>BM-MNCs</td>
<td>Trans-endocardial</td>
<td>3</td>
<td>↓ Angina, Stress ischemia</td>
</tr>
<tr>
<td>Tse et al.</td>
<td>2003</td>
<td>UC</td>
<td>8</td>
<td>BM-MNCs</td>
<td>Trans-endocardial</td>
<td>3</td>
<td>↑ MP, LVWM</td>
</tr>
<tr>
<td>Perin et al.</td>
<td>2004</td>
<td>NR</td>
<td>11</td>
<td>BM-MNCs</td>
<td>Trans-endocardial</td>
<td>12</td>
<td>↑ Myocardial O2 consumption</td>
</tr>
<tr>
<td>Silva et al.</td>
<td>2004</td>
<td>NR</td>
<td>5</td>
<td>BM-MNCs</td>
<td>Trans-endocardial</td>
<td>6</td>
<td>↑ Myocardial O2 consumption</td>
</tr>
<tr>
<td>Kuethe et al.</td>
<td>2005</td>
<td>UC</td>
<td>5</td>
<td>BM-MNCs</td>
<td>Intracoronary</td>
<td>3,12</td>
<td>No improvement</td>
</tr>
<tr>
<td>Bartuneck et al.</td>
<td>2005</td>
<td>NR</td>
<td>19</td>
<td>CD133+ BM-EPCs</td>
<td>Intracoronary</td>
<td>4</td>
<td>↑ LVEF, MP</td>
</tr>
</tbody>
</table>

CNSAS, Canadian Cardiac Association Angina Score; CFR, coronary flow reserve; DB, double blinded; IS, infarct size; LVESV, left ventricular end systolic volume; LVWM, left ventricular wall motion; MP, myocardial perfusion; NR, nonrandomized; NYHA, New York Heart Association class; RC, randomized controlled; RD, reversible defect; R, randomized; SV, stroke volume; UC, uncontrolled.
defect and improvement in global LV function on quantitative single-photon emission computed tomography analysis compared with a nonrandomized contemporary control group. At 4 months, enhanced LVEF and reduced end-systolic volume were detected by LV angiogram and electromechanical mapping. These encouraging results were followed by a similarly designed study by Perin (2004), including 11 CHF patients, with an extended follow-up period of 12 months. Myocardial perfusion and exercise capacity were significantly improved, while the increase in LVEF failed to be reproduced. Furthermore, histological examination performed 11 months after cell injection showed no irregular cell growth or tissue lesions and revealed the presence of an active process of angiogenesis within the fibrotic tissue and the adjacent myocardium. This procedure was repeated also in a small cohort of 5 cardiac transplant candidates by Silva et al. (2004) and yielded a major increase in myocardial oxygen consumption, to the extent that in 4 of the 5 cases, the patients were no longer eligible for cardiac transplantation. Noteworthy complications including significant arrhythmias were not evident during each of these trials.

Additional nonrandomized trials (Fuchs et al., 2003; Tse et al., 2003) evaluated the efficacy of Percocutaneous transcendocardial delivery of autologous BM-MNCs in “no-option” patients with refractory angina and myocardial ischemia. After 3 months, the authors reported improvements in Canadian Cardiovascular Society angina score, stress-induced ischemia as well as in target wall motion and thickening. During a relatively long-term follow-up (12–36 months), clinical improvement was sustained although some patients had undergone revascularization procedures. Moreover, no tumor formation, intramyocardial calcification, ventricular arrhythmia, or sudden death was observed.

Trans-epicardial injections have been employed by Stamm et al. (2003) for intramyocardial implantation of purified CD133 BM-derived progenitors in 6 coronary artery bypass grafting (CABG)-treated patients. During the intervention, cells were injected directly along the circumference of the infarct border. Three to nine months after surgery, global LV function was enhanced in 4 patients and infarct tissue perfusion was markedly improved in 5 subjects. When last seen (9–16 months after surgery), none of the patients had malignant neoplasia or ventricular arrhythmia and all reported a notable improvement in daily activities.

Finally, Tateishi-Yuyama et al. (2002) tested the feasibility and safety of autologous implantation of BM-MNCs in patients with ischemic limbs due to PVD. During this study, 22 recruited patients, suffering from bilateral leg ischemia, were randomly injected intramuscularly with BM-MNCs in one leg and PB-MNCs in the other as a control. At 4 weeks, ankle-brachial index was significantly improved in legs injected with BM-MNCs compared with those injected with PB-MNCs. Similar improvement was observed for transcutaneous oxygen pressure, rest pain, and pain-free walking time. These improvements were sustained at 24 weeks, suggesting that this strategy could be safe and effective for the accomplishment of therapeutic angiogenesis in PVD.

6.2.3. Progenitor cell mobilization

Various cytokines have been shown to induce EPC mobilization from the BM. Animal experiments have additionally demonstrated reduced myocardial necrosis and infarct size after G-CSF treatment (Minatoguchi et al., 2004). Following this, systemic administration of cytokines inducing EPC mobilization has also been investigated in an attempt to circumvent the invasive interventions required for cell transplantation. In this context, numerous clinical studies have been established in order to explore the feasibility and safety of G-CSF therapy for the treatment of CV diseases (Table 2).

In a randomized study (Valgimigli et al., 2005) including 20 patients after MI, subjects were treated either by G-CSF treatment (5 μg/kg/day s.c. for 4 days) or placebo. G-CSF was found to markedly increase PB CD34+ cells and CD34+/CD133+/VEGFR2+ EPCs compared with placebo. At 6 months follow-up, LVEF and especially LV end diastolic volume tended to be relatively higher in the G-CSF-treated group. The FIRSTLINE-AMI (front-integrated revascularization and stem cell liberation in

### Table 2
Clinical trials: G-CSF for progenitor cell mobilization

<table>
<thead>
<tr>
<th>Group/study</th>
<th>End point year</th>
<th>Design</th>
<th>No. of treated patients</th>
<th>Treatment</th>
<th>Main cells mobilized</th>
<th>Follow up (months)</th>
<th>Main outcomes (compared with controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuethe et al.</td>
<td>2004</td>
<td>UC</td>
<td>5</td>
<td>G-CSF (10 μg/kg/day s.c. for 7 days)</td>
<td>CD34+</td>
<td>3</td>
<td>↑ LVEF, LVWM, MP</td>
</tr>
<tr>
<td>Kuethe et al.</td>
<td>2005</td>
<td>NR</td>
<td>14</td>
<td>G-CSF (10 μg/kg/day s.c. for 7 days)</td>
<td>CD34+</td>
<td>3</td>
<td>↑ LVEF, LVWM, MP</td>
</tr>
<tr>
<td>Valgimigli et al.</td>
<td>FIRST LINE-AMI</td>
<td>2005</td>
<td>RC</td>
<td>G-CSF (5 μg/kg/day s.c. for 4 days)</td>
<td>CD34+ and CD34+/CD133+/VEGFR2+ MN-CD34+</td>
<td>3, 6</td>
<td>No further improvement</td>
</tr>
<tr>
<td>Wang et al.</td>
<td>2005</td>
<td>C</td>
<td>13</td>
<td>G-CSF (10 μg/kg/day s.c. for 6 days)</td>
<td>CD34+</td>
<td>1, 2, 6</td>
<td>↓ LVR</td>
</tr>
<tr>
<td>STEMMI</td>
<td>2006</td>
<td>R,PC,DB</td>
<td>38</td>
<td>G-CSF (10 μg/kg/day s.c. for 6 days)</td>
<td>leukocytes and CD34+</td>
<td>1, 6</td>
<td>↓ Angina, ↓ NTG consumption</td>
</tr>
<tr>
<td>REVIVAL-2</td>
<td>2006</td>
<td>R,PC,DC</td>
<td>56</td>
<td>G-CSF (10 μg/kg/day s.c. for 5 days)</td>
<td>leukocytes and CD34+</td>
<td>4, 6</td>
<td>No further improvement</td>
</tr>
</tbody>
</table>

DB, double blinded; IS, infarct size; LVR, left ventricular remodeling; LVWM, left ventricular wall motion; MN, mononuclear; MP, myocardial perfusion; NR, nonrandomized; NTG, nitroglycerine; RC, randomized controlled; UC, uncontrolled.

Please cite this article as: Ben-Shoshan, J., & George, J. Endothelial progenitor cells as therapeutic vectors in cardiovascular disorders: From experimental models to human trials. *Pharmacol Ther* (2007), doi:10.1016/j.pharmthera.2007.03.012
evolving AMI by use of G-CSF) was aimed to evaluate the impact of G-CSF integrated with primary PCI of AMI (Ince et al., 2005). Among 50 recruited patients, 25 patients were randomly assigned to receive G-CSF (10 μg/kg/day s.c. for 6 days) in addition to standard care. Within 4 months, the treated group showed improved LV dimensions, EF, and wall-motion. However, those 2 studies were not blinded and the follow-up results were available from only 14 and 11 patients, respectively.

Despite encouraging results, 2 large recent cohort studies (Ripa et al., 2006; Zohlnhofer et al., 2006) have undermined the potential capacity of G-CSF treatment to improve cardiac function. During the STEM-MI (stem cell mobilization induced by subcutaneous G-CSF to improve cardiac regeneration after acute ST-elevation MI) and REVIVAL-2 (regenerate vital myocardium by vigorous activation of BM stem cells) studies, 72 and 114 patients after MI and successful reperfusion by primary PCI entered a randomized, double-blind, placebo-controlled trial, respectively. Patients were randomly assigned to receive either a daily dose G-CSF treatment (10 μg/kg/day s.c. for 5–6 days) or placebo. At the 6 months end point, both investigators concluded that G-CSF did not lead to further recovery of ventricular function or infarct size compared with placebo.

Few studies explored the combination of G-CSF administration and subsequent infusion of the collected progenitor cells-enriched PB-MNCs. In 1 study (Ozbaran et al., 2004), 6 patients diagnosed for ischemic cardiomyopathy were treated with G-CSF (30 × 10⁶ i.u./day s.c. for 4 days). PB-MNCs were then collected by apheresis and implanted into areas of myocardial injury during standard cardiopulmonary by-pass procedure. After 4 months, improved cardiac function was documented by echocardiography, thallium scintigraphy, and photon emission tomography. In the MAGIC trial (Kang et al., 2004), 27 patients with MI who underwent coronary stenting were prospectively randomised into 3 groups; G-CSF followed by cell infusion, G-CSF alone, and control group. At 6 months, exercise capacity, myocardial perfusion, and systolic function were improved in patients treated with G-CSF and cell infusion, compared with the other groups. Unexpectedly, an elevated rate of in-stent restenosis was observed in patients that received G-CSF.

7. Potential hurdles and adverse effects

Several key barriers will probably have to be overcome, before EPC-based therapy could be clinically implemented. First, EPC phenotype definition will have to be further elucidated in order to enable better interpretation of the results achieved by different study.

The impact of EPC transfer on formation and progression of atherosclerotic plaques still remain controversial. Rauscher et al. (2003) found that treatment with BM-derived EPCs from young ApoE−/− mice prevent plaque progression in adult atherosclerotic ApoE−/− recipients. On the other hand, using age-matched ApoE−/− mice model, we have recently showed that intravenous transfer of total BM cells or EPCs increase atherosclerotic lesion size and influenced plaque stability (George et al., 2005a). Our findings are consistent with the data demonstrating that BM-derived cells may give rise to smooth muscle cells and contribute to pathological atherosclerotic vessel wall remodeling (Caplice et al., 2003). However, the relatively large number of cells we used for transfer could have accounted for the proatherogenic effects. Another recent work described the appearance of severe calcification following intramyocardial transplantation of unselected BM cells in a rat MI model (Yoon et al., 2004). Interestingly, calcification was absent in animals receiving the same number of clonally expanded BMCs. Moreover, EPCs were found to contribute to neointimal lesions in the microvessels of murine aortic allografts, suggesting the involvement of EPCs in allograft vasculopathy (Hu et al., 2003). This finding, however, was challenged by a later study (Hillebrands et al., 2003). These equivocal findings highlight the importance of a more accurate phenotypic characterization and delineation of a delivery route of the transferred cells.

The interpretation of the clinical trials are so far limited by several facts. First, a large part of these trials lacked appropriate controls, randomization, and blinding. Secondly, most studies were conducted on small numbers of patients followed-up for short periods. Furthermore, distinct cell populations and routes of delivery were often used, making cross-sectional comparison difficult. Eventually, the tendency of cardiac function to improve with time following well treated MI complicate the evaluation of the true contribution of cell therapy.

It will be necessary to verify that during the follow-up periods of the above mentioned clinical trials, cell transfer or mobilization were not associated with consistent untoward adverse events, including proarrhythmic effects, myocardial calcification, or aggravation of heart failure. During the MAGIC trial Kang et al. (2004) reported that the improvement in systolic function was accompanied by a high rate of in-stent restenosis in 7 of 10 G-CSF treated patients, leading to cessation of the trial. The investigators hypothesized that BMC differentiation into smooth muscle cells within the stented segment might have produced increased restenosis rate. However, in the larger STEM-MI study, Ripa et al. (2006) concluded that G-CSF treatment, following coronary stent implantation in primary PCI-treated AMI patients, does not increase in-stent restenosis rate excessively. This last report was supported by the FIRSTLINE-AMI and REVIVAL-2 studies.

Finally, a crucial concern with any proangiogenic therapy, such as EPCs transfer or cytokine administration, is the development of malignant angiogenic neoplasia or, alternately, the vascularization of occult tumors. Careful precautions should be taken in this context, considering the growing evidence suggesting that EPCs actually contribute to tumor neovascularization (Davidoff et al., 2001). In this context it is important to mention that no angiogenic neoplasia has been reported in any of the clinical trials reported so far.

8. Future prospective

Despite the remarkable advances made during the last decade in understanding EPC biology, the precise mechanisms of migration, homing, and differentiation has yet been fully clarified. Further investigation is required in order to improve the isolation, identification, and enrichment of EPCs. Definitive characterization of EPC phenotype will permit the standardization of EPC...
purification protocol, which is of major importance for clinical applications.

Recently, the cellular interactions and paracrine effects of EPCs have also found to play a role of major interest in their contribution the vasculogenic process. For instance, different adhesion molecules and matrix proteins were shown to be required for the integration of circulating EPC into ischemic tissue and to subsequent neovascularization (Chavakis et al., 2005; Koyanagi et al., 2005; Yoon et al., 2006). Moreover, EPCs have been demonstrated to secrete a variety of growth factors, which support the survival and function of tissue residing cells, such as mature endothelial cells and cardiac progenitors, thereby, accelerating the process of new vessel formation and organ regeneration (Urbich et al., 2005). Further understanding of these interactions will allow improved prediction of the behavior of injected progenitors within ischemic tissues.

Ultimately, different novel strategies are being integrated in order to improve the function and viability of EPCs. Gene modifications using viral vectors have been employed to improve the angiogenic properties of EPCs, as well as to use EPCs as vehicles for delivery of therapeutic factors. Accordingly, over-expression of VEGF in EPCs was found to promote EPC proliferation and angiogenic capacity (Iwaguro et al., 2002). EPCs overexpressing anticoagulant and vasculoprotective genes were found to reduce pathological vascular remodeling more efficiently compared with regular EPCs (Griese et al., 2003; Kong et al., 2004). Gene manipulation strategies are therefore likely to enhance the efficacy of cell based therapy.

Biodegradable scaffolds seeded with BMCs were demonstrated by Matsumura et al. (2003) to secret factors which promote homing, adhesion, and proliferation of circulating EPC, resulting in the development of a new vessel wall. Moreover, deployment of EPC-covered intravascular stents has been suggested for prevention of stent thrombosis and restenosis, as well as for rapid formation of normal tissue architecture (Shirota et al., 2003). Finally, another novel approach to therapeutic modulation of EPCs is the employment of stents coated with anti-CD34 antibodies to capture circulating EPCs within the injured vessel segment (Aoki et al., 2005).

9. Summary

- Adult bone marrow is a source of EPCs capable of homing to sites of neovascularization and incorporating into growing vessels.
- Circulating EPC numbers and functional properties are compromised in various CV disorders and could potentially set them as diagnostic surrogate markers.
- A wide range of cytokines, growth factors, hormones, and pharmacological agents have been found to promote EPC mobilization from the bone marrow, suggesting this process as feasible proangiogenic therapeutic strategy.
- Experimental animal studies have demonstrated the beneficial potential of EPC-based cell therapy for the treatment of ischemic vascular disorders.
- Pioneering small clinical trials employing progenitor cells mobilization and implantation have shown equivocal results regarding the effectiveness of progenitor cell therapy in restoring cardiac function.
- Additional large-scale, randomized, and controlled trials as well as a more profound understanding of EPC biology are required to truly assess the safety and clinical potential of this novel therapy.
- Safety issues regarding progenitor cell therapy will also have to be more thoroughly addressed by large clinical trials.

References


J. Ben-Shoshan, J. George / Pharmacology & Therapeutics xx (2007) xxx–xxx

Please cite this article as: Ben-Shoshan, J., & George, J. Endothelial progenitor cells as therapeutic vectors in cardiovascular disorders: From experimental models to human trials. Pharmacol Ther (2007), doi:10.1016/j.pharmthera.2007.03.012

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