Brief Reviews

Mobilizing Endothelial Progenitor Cells

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Abstract—Mobilization of endogenous endothelial progenitor cells (EPCs) from the bone marrow may be an alternative way to increase neovascularization and may be used as therapeutic option for the treatment of ischemic cardiovascular diseases. In this review, we discuss the EPC mobilizing effects of pro-inflammatory cytokines such as granulocyte monocyte colony-stimulating factor and granulocyte colony-stimulating factor, growth factors such as vascular endothelial growth factor, placental growth factor, erythropoietin, and angiopoietin-1, chemokines such as stromal cell–derived factor-1, hormones such as estrogens and lipid-lowering and anti-diabetic drugs, as well as physical activity. (Hypertension. 2005;45:321-325.)

Key Words: cardiovascular diseases

In recent years, our understanding of the processes responsible for the formation of new blood vessels after tissue ischemia has changed. The vascularization of ischemic tissue in adults was once thought to be restricted to migration and proliferation of mature endothelial cells, a process termed “angiogenesis.” However, increasing evidence suggests that stem cells are mobilized from the bone marrow into the circulation, differentiate in circulating endothelial progenitor cells (EPCs), and home to sites of ischemia to contribute to the formation of new blood vessels. In analogy to the embryonic development of blood vessels from primitive endothelial progenitors (angioblasts), this process is referred to as “vasculogenesis.” Endothelial progenitors have been derived from more differentiated CD34+ or immature CD133+ hematopoietic stem cells, as well as from peripheral blood mononuclear cells or CD14+ monocytes. Although EPCs can be generated from different sources, they all showed expression of endothelial marker proteins such as vascular endothelial growth factor (VEGF) receptor 2 (KDR), von Willebrand factor, and endothelial nitric oxide synthase (eNOS). The potency of circulating progenitor cells is demonstrated by the fact that intravenous infusion of bone marrow–derived stem and progenitor cells augments neovascularization in vivo. Application of either bone marrow–derived or circulating blood–derived progenitor cells into the infarct artery beneficially affects postinfarction remodeling.

Mobilization of EPCs for Improvement of Neovascularization

Mobilization of endogenous EPCs from the bone marrow may be an alternative way to increase postnatal neovascularization. Endogenously, VEGF and stromal cell–derived factor-1 (SDF-1), which are produced by ischemic areas, seem to have important roles for mobilization of EPCs (Figure). Increasing the levels of circulating VEGF by using plasmids or recombinant protein also enhanced the levels of circulating EPCs in experimental models, as well as in clinical pilot trials. Moreover, SDF-1 or angiopoietin-1 overexpression by using adenovirus-mediated gene delivery increased EPC levels in murine models. Additional cytokines mobilizing EPCs and hematopoietic progenitor cells include granulocyte monocyte colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF), with the latter being used for bone marrow transplantation in the clinical setting for years. Particularly, G-CSF also promotes inflammation by inducing a profound increase in the number of circulating leukocytes. Because inflammation plays a key role for the development of atherosclerotic lesions and restenosis, as well as plaque instability leading to acute coronary syndromes, recent studies questioned the safety of G-CSF application in patients with acute or chronic myocardial infarction. Although the increased restenosis rate might not be exclusively caused by the application of G-CSF but also may have been influenced by the study design (no initial percutaneous coronary intervention), the use of mobilizing factors with a lower proinflammatory effect might be preferable in patients with coronary artery disease.

An alternative cytokine with a lower proinflammatory profile is erythropoietin (Epo). One of the main functions of the cytokine Epo is to stimulate the proliferation of early erythroid precursors and the differentiation of later precursors of the erythroid lineage. However, Epo has recently been shown to perform more functions than erythropoiesis. Moreover, mature endothelial cells also express Epo receptors and Epo increased the number of endothelial cells in vitro. This responsiveness of both vascular and hematopoietic systems reflects the common ontogenesis of endothelial and
hematopoietic cells, suggesting that both cell lineages share a common progenitor, the hemangioblast. Consistently, Epo significantly increased mobilization of circulating EPCs in experimental models in vivo\(^2\) and stimulated the mobilization of CD34\(^+\)/CD45\(^+\) circulating EPCs in peripheral blood in humans.\(^2\) Likewise, our human studies identify Epo as an independent predictor of CD34\(^+\)/KDR\(^+\) EPC number and function in patients with coronary heart disease.\(^2\) The correlation between Epo serum levels and the number of CD34\(^+\) or CD133\(^+\) hematopoietic stem cells in the bone marrow in patients with ischemic coronary artery disease further supports an important role of endogenous Epo levels. These data suggest that Epo is an important physiological determinant of EPC mobilization. Epo elicits a similar potency for the improvement of EPC mobilization as VEGF. In addition, Epo is also protective for cardiac myocytes after ischemia/reperfusion in patients with heart failure, because it decreases the number of apoptotic myocytes, thereby limiting infarct expansion and attenuating the postinfarct deterioration in hemodynamic function.\(^2\) Moreover, anemia has been recognized as an important comorbid condition in patients with heart failure. These beneficial effects of Epo may override potential Epo-related side effects such as elevated blood pressure and the incidence of thrombosis.\(^2\)

In addition to the use of cytokines to mobilize EPC from the bone marrow, several pharmacological substances have been shown to increase EPC numbers. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, or statins, have been developed as lipid-lowering drugs, but besides lipid-lowering, statins are capable of reducing vascular inflammation, decreasing platelet aggregation and thrombus deposition, and increasing endothelium-derived nitric oxide (NO) production. Moreover, statins induced mobilization of EPCs from the bone marrow.\(^2\)\(^6\)\(^7\) This was demonstrated by using atorvastatin to increase the number of spleen-derived EPCs,\(^7\) as well as rosvastatin that enhanced scala1\(^-\)VEGF-R2 (flk-1)\(^+\) EPCs in the circulation.\(^2\)\(^8\) These results were confirmed by the demonstration that statins augmented incorporation of EPCs mobilized from the bone marrow into foci of corneal neovascularization.\(^2\)\(^6\) Statins also stimulated EPC mobilization and neovascularization in mice after myocardial infarction and improved left ventricular function.\(^2\)\(^9\) Likewise, in patients with stable coronary artery disease, treatment with a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor also augmented circulating endothelial progenitor cells, comparable to patients undergoing VEGF gene transfer for ischemia.\(^2\)\(^7\) Recently, in fact, statin therapy has been shown not only to rapidly enhance coronary blood flow in patients with stable coronary artery disease but also to reduce myocardial ischemia in patients with acute coronary syndromes within a few weeks of treatment.

The increase of circulating EPC numbers by statin treatment requires the eNOS.\(^2\)\(^0\) eNOS contributes to blood vessel relaxation in the periphery and is essential in the bone marrow microenvironment.\(^3\)\(^0\) The regulatory components of the bone marrow microenvironment, the osteoblasts and endothelial cells, express the eNOS and release eNOS-derived NO to allow for mobilization of EPC and hematopoietic progenitor cells.\(^3\)\(^0\) Because statins profoundly augment eNOS expression and activity,\(^3\) one may speculate that statins increase the concentration of eNOS-derived NO in the bone marrow.\(^3\)\(^0\)\(^3\)\(^1\)\(^3\)\(^2\)

Similar to statins, the antidiabetic and anti-inflammatory peroxisome proliferator-activated receptor-\(\gamma\) agonists promote differentiation and mobilization of angiogenic progenitor cells and improve re-endothelialization after vascular intervention.\(^3\)\(^3\)

Recently, physical exercise, an important atheroprotective factor, was shown to enhance circulating EPC levels.\(^2\)\(^8\)\(^3\)\(^5\) The mechanisms by which exercise increase EPC levels are not entirely clear. One may speculate that the induction of ischemia in the muscles enhances circulating cytokine levels. Alternatively, a direct effect of increased blood flow in the bone marrow might be relevant. The latter possibility is underscored by the fact that mice lacking eNOS did not show an augmented EPC mobilization after exercise.\(^2\)\(^8\) Finally, estrogen application increased EPC levels in mice.\(^3\)\(^6\)\(^3\)\(^7\)

**Mechanisms of EPC Mobilization**

The local bone marrow microenvironment, the so-called stem cell niche consisting of fibroblasts, osteoblasts, and endothelial cells, governs the maintenance and mobilization of bone marrow stem cells.\(^3\)\(^2\)\(^3\)\(^8\)\(^3\)\(^9\) Mechanistically, cytokines inducing mobilization interfere with the interactions between stem cells and bone marrow stromal cells, which allow stem cells to disengage the bone marrow, and to pass through the sinusoidal endothelium to enter the blood stream. Stem cell mobilization is mediated by proteinases such as elastase, cathepsin G, and matrix metalloproteinases (MMPs).\(^4\) A cytokine clinically used for the mobilization of CD34\(^+\) cells in patients is G-CSF, which releases the proteinases elastase and cathepsin G from neutrophils. These proteinases induce cleavage of adhesive bonds on stromal cells, which interact with integrins on hematopoietic stem cells.\(^4\)\(^1\) Moreover, these proteinases cleave the cytokine SDF-1, which is released by stromal cells and its receptor CXCR4 on stem and progenitor cells.\(^4\) Stem cell mobilization as a result of high levels of circulating SDF-1 appears to reverse the SDF-1 gradient across the bone marrow barrier, forcing CXCR-4\(^+\) cells to exit the bone marrow.\(^4\)\(^3\) However, VEGF, SDF-1, and placental growth factor (PIGF)-induced stem cell mobilization was shown to rely on MMP-9.\(^4\)\(^4\)\(^4\) When PIGF is administered in the early phase of bone marrow recovery, it is chemoattractive for VEGF-receptor-1\(^+\) stem cells, whereas in later stages PIGF functions are mediated by MMP-9.\(^4\)\(^5\) Thus, increasing the local concentration of MMP-9 in the bone marrow cleaves membrane bound Kit ligand (mKitL) and,
finally, releases soluble Kit ligand (KitL; also known as stem cell factor). After all, this process transfers endothelial and hematopoietic progenitor cells from the quiescent to the proliferative niche.

However, the question of whether G-CSF-induced stem cell mobilization depends on MMP-9 is still a matter of debate. This controversy might be explained by the fact that MMP-9 plays a pivotal role in growth factor-induced hematopoietic progenitor mobilization in wild-type animals, whereas compensatory upregulation of enzymes with a similar activity profile to MMP-9 might mask the impact of MMP-9 deficiency in the knockout model.

As discussed, eNOS is essential to maintain adequate progenitor cell mobilization in response to distinct stimuli, including VEGF, statins, exercise, and estrogen, in the regulation of stem and progenitor cell mobilization. The defective mobilization was caused by the lack of eNOS (Nos3) provided by the bone marrow stromal microenvironment. This was demonstrated in Nos3−/− mice that have undergone wild-type stromal cell–free bone marrow transplantation, which still had a blunted mobilization, although wild-type stem cells efficiently engrafted within the bone marrow. Interestingly, after Nos3−/− stromal cell–free bone marrow transplantation into wild-type animals, mobilization occurred as a result of the functional wild-type microenvironment into which the Nos3−/− hematopoietic cells were transplanted. Therefore, eNOS deficiency in the bone marrow microenvironment impaired the mobilization of stem and progenitor cells from the bone marrow. In contrast, intravascular injection of stem and progenitor cells circumvented the defective mobilization from the bone marrow and improved the neovascularization after induction of hind limb ischemia. Therefore, eNOS-derived NO is a physiological regulator of stem and progenitor cell mobilization in the bone marrow stromal microenvironment.

To investigate the mechanisms that are required for eNOS-mediated stem and progenitor mobilization, we investigated expression of MMP-9, which is important for the bone marrow microenvironment. We demonstrated that Nos3−/− mice have a profoundly reduced basal activity of pro-MMP-9 and that mobilization-induced MMP-9 is greatly decreased in the absence of eNOS. As a consequence, sKitL release from mKitL is reduced in Nos3−/− mice. Therefore, hematopoietic recovery was decreased in Nos3−/− mice, which resembled the phenotype of MMP-9−/− mice. Of note, the failure of Nos3−/− mice to mobilize EPC was rescued by the infusion of sKitL, which bypasses the requirement for MMP-9–mediated cleavage of mKit. Thus, these studies established eNOS activation as a novel mechanistic link between VEGF signaling and MMP-9 expression in bone marrow vascular stroma.

**Potential Adverse Effects of Stem and Progenitor Cell Mobilization**

Adverse effects of EPC mobilization have been described as contribution of EPCs to tumor neovascularization in some tumor models. Moreover, circulating progenitor cells have been implicated in the neovascularization of atherosclerotic lesions of athero-rafts and in further atherosclerotic plaque progression in an ischemic setting. However, transfection of EPCs enhanced re-endothelialization and reduced neointima formation after vascular injury. One may speculate that the endothelial repair capacity might override the potential harmful effects of plaque neovascularization. Thus, future studies have to determine the overall influence of EPC levels on atherosclerotic disease progression and prognosis.

**Open Questions**

Mobilization of EPC may be a possible novel therapeutic option to enhance endothelial regeneration and neovascularization. Experimental studies showed a significant enhancement of neovascularization by factors that systemically increase EPC levels.

However, various open questions need to be addressed in the future. The use of mobilizing cytokines at present is hampered by the fact that most powerful mobilizers such as G-CSF also exert a proinflammatory capacity, which may enhance atherosclerotic disease progression in patients with coronary artery disease. In contrast, statins or exercise appear to more selectively enhance EPC levels and does not provoke a pro-inflammatory action on the vascular wall. Although ample experimental studies and clinical trials have demonstrated a protective function of statins or exercise, it has to be determined whether this is mediated by the moderate augmentation of EPC. Statins, exercise and other factors, eg, Epo, also directly act on mature endothelial cells and may facilitate endogenous repair by >1 mechanism.

A second open question is whether the systemic increase in circulating EPC levels is preferable compared with a local infusion or injection of ex vivo isolated bone marrow or circulating cells to augment neovascularization.

Finally, the requirement of eNOS for VEGF, statin, exercise, and estrogen-mediated mobilization of EPC raises the question whether the mobilization capacity of patients with coronary artery disease is impaired. Given that patients with coronary artery disease showed a diminished NO bioavailability in peripheral endothelial cells, one may speculate that this also translates into the bone marrow. EPC numbers are lower in patients with coronary artery disease or diabetes and are correlated with NO-dependent vasorelaxation measured in the forearm.

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