Beneficial Effects of Statins on Endothelial Progenitor Cells

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Abstract: In recent years, endothelial progenitor cells (EPCs) have been demonstrated to play an important role during tissue vascularization and endothelium homeostasis in adults. In addition, EPCs have been implicated in the pathophysiology of cardiovascular and cerebrovascular disease, such that a decreased number of EPCs may not only be a risk indicator but also a potential therapeutic target. Of the many agents that have been examined to increase EPCs and enhance their function, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors or statins are one of the most intriguing. Accumulated evidence has demonstrated that statins promote EPC mobilization, proliferation, migration, adhesion, differentiation and reduce senescence and apoptosis independent of their serum lipid-lowering effect. This review summarizes the understanding of current mechanisms explaining the myriad of beneficial effects of statins on EPCs and discusses future challenges for studies involving statins and subpopulations of EPCs. However, the pharmacologic mechanisms of action of statins on EPCs remain at the cellular level, whereas the putative molecular mechanisms await further studies.

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In 1997, Asahara et al[1] first defined, isolated, characterized and examined the *in vivo* function of putative endothelial progenitor cells (EPCs) from human peripheral blood. Since then, numerous basic science and clinical studies have continued to characterize these cells. The number of EPCs is related to many diseases (Table 1). The relationship between EPCs and cardiovascular disease remains a main research interest. Previous work has demonstrated that there is a significant inverse relationship between the EPC number and the Framingham risk factor score.[2] Other studies involving healthy men[3] or patients with coronary artery disease (CAD)[4–6] have shown that levels of EPCs may be a surrogate biologic marker for endothelial function, CAD severity[7] and cumulative cardiovascular risk. In addition, data have shown that the number of circulating EPCs increases in patients with cardiovascular events, such as unstable angina and myocardial infarction.[8] In heart failure, EPC mobilization has shown a biphasic response, with elevation in early stages, whereas in class IV, their peripheral mobilization is reduced not only with respect to classes II and I but also with respect to controls.[9] An exhaustion of EPCs in the advanced phases of the disease may be one explanation for this phenomenon.[9] Furthermore, 2 independent prognostic studies have demonstrated that lower EPC numbers are related to a higher cerebrovascular event rate.[10] A study performed by Lapergue et al[11] demonstrated that EPC counts in patients were significantly reduced among a stroke population compared with a control group ($P = 0.003$). In another study involving 88 subjects, Ghani et al[2] demonstrated that the number of circulating EPCs was significantly lower in patients with cerebrovascular disease compared with control subjects, suggesting that decreased EPC numbers were a risk marker for future cerebrovascular events. In patients with cardiovascular and cerebrovascular risk factors, including hyperlipidemia,[8] hypertension,[8] diabetes mellitus,[8] metabolic syndrome,[12] obesity,[13] aging[14] and smoking,[15] there were reduced numbers and impaired functionality of EPCs. The cumulative evidence indicates that a low EPC count is a potential risk factor for cardiovascular or cerebrovascular disease.

It is clear that EPCs are recruited to sites of injury, participate in the repair of damaged tissues, form new vessels to facilitate recovery after ischemic events and help attenuate the development and progression of atherosclerosis.[8,10,14] Therefore, it has been speculated that enhancing the endothelial regenerative capacity by EPC administration or mobilization represents a novel therapeutic approach in both cardiovascular and cerebrovascular diseases. Several preclinical studies have already demonstrated the therapeutic efficacy of EPCs in ischemic disorders and vascular injury in animal models.[8] In a rabbit model of carotid artery denudation, transplantation of autologous EPCs led to enhanced re-endothelialization of blood vessels 4 weeks after the injury and also improved endothelial function of the denuded carotid artery.[11] In another small animal model (athymic nude rats) of hind limb ischemia, direct injection of human circulating EPCs improved neovascularization and subsequent limb blood flow.[16] In a balloon injury model, after mobilization of circulating EPCs, the accelerated repair of the denuded endothelium along with the decreased activation of medial smooth muscle cells and neointima formation occured.[17] In addition, studies using various myocardial ischemia models demonstrated similar results with a subsequent improvement in myocardial perfusion and left ventricular function.[16] Based on this encouraging preclinical work, an increasing number of small-scale clinical trials have been performed with promising results. These include directly injecting EPCs into infarcted tissue or injecting proangiogenic cytokines into a target tissue to increase EPC number and function. Dobert et al[18] demonstrated that intracoronary infusion of bone marrow-derived (15 patients) or circulating blood-derived EPCs (11 patients) 4 days after acute myocardial infarction resulted in a significant increase in myocardial viability and perfusion, which were reliably measured by ^{18}F-fluorodeoxyglucose positron emission tomography and ^{201}Tl single-photon emission computed tomography. Another study involving patients who had a symptomatic New York Heart Association scale of at least II and an ejection fraction less than 35% demonstrated significant improvement 24 months after treatment with intracoronary infusion of autologous EPCs after granulocyte-colony stimulating factor mobilization.[19] In patients with stable CAD, similar results were found.[20] Collectively, these animal experiments and clinical trials strongly support the notion that enhancement of EPC number or function is a viable therapeutic approach.

Not surprisingly, pharmacological agents that can improve the number or function of EPCs have become an area of great interest. Several clinically used pharmacological

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TABLE 1. Human diseases or states associated with altered circulating EPC number and function

Lower	Higher
Hyperlipidemia[8]	Exercise[55]
Hypertension[8]	Intake of red wine[56]
Diabetes mellitus[8,57]	Unstable angina[8]
Metabolic syndrome[12]	Myocardial infarction[8]
Obesity[13]	
Aging[14]	
Smokers[15]	
Stable CAD[6]	
Cerebrovascular atherosclerosis[8]	
Stroke[2]	
Chronic pulmonary diseases[58]	
Chronic renal insufficiency[10]	
Hyperhomocysteinemia[10]	
Rheumatoid arthritis[10,59]	
Avascular necrosis of the femoral head[60]	
Inflammatory bowel disease[61]	

EPC, endothelial progenitor cells; CAD, coronary artery disease.

agents, cytokines, growth factors and hormones have been examined for their ability to promote EPC-mediated endothelial repair in experimental studies[21] (Table 2). Among these factors, statins have become an important drug class. Besides their ability to reduce serum cholesterol levels, they have pleiotropic effects in many diseases. The details of statin pleiotropy are listed in Table 3; all of the additional therapeutic effects are independent of their lipid-lowering benefits.[22] The possible mechanisms by which these drugs may modulate EPCs will be extensively reviewed later.

TABLE 2. Pharmaceutical agents that increase number and functional activity of EPCs

Statins[21]
ACEIs[21]
ARBs[21,62,63]
Dihydropyridine calcium channel blockers[21,64]
β-blockers: carvedilol and nebivolol[21]
PPAR-γ agonists: rosiglitazone and pioglitazone[21,65]
Insulin[21]
Erythropoietin[21]
VEGF[11]
G-CSF[10]
Estrogens[8]
ADMA, an endogenous NOS inhibitor[8]
Vardenafil, a phosphor-diesterase inhibitor[8]
Puerarin[8]
Ginkgo biloba extract[8]
Proteasome inhibitor MG132[66]
Neurotrophic factors[67]
Heme oxygenase-1[68]
B-type natriuretic peptide[69]
Sphingosine kinase[70]

EPCs, endothelial progenitor cells; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin II type 1 receptor antagonists; VEGF, vascular endothelial growth factor; ADMA, asymmetric dimethylarginine; NO, nitric oxide; G-CSF, granulocyte colony stimulating factor; PPAR-γ, peroxisome proliferator-activated receptor-γ .

TABLE 3. Pleiotropic effects of statins

Anti-inflammatory effect[22,71,72]
Antioxidant[73,74], decrease oxidative stress[22]
Stabilization of atherosclerotic plaque[22,75]
Diminish blood viscosity and thrombin generation[22]
Inhibit proliferation and migration of vascular smooth muscle cells[22]
Improve endothelial function[22]
Immunomodulatory effect[23,73]
Antiapoptotic features[73]
Antithrombotic effect[73]
Antimicrobial effect[73]
Slow the rate of cognitive decline and delay the onset of AD and all-cause dementia in cognitively healthy elderly individuals[76]
Neuroprotection[23]

CD, artery disease.

POSSIBLE MECHANISMS EXPLAINING THE EFFECTS OF STATINS ON EPCS

Generally, by inhibiting the conversion of 3-hydroxy-3-methylglutaryl coenzyme A to L-mevalonic acid, statins prevent the synthesis of important isoprenoids, such as farnesylpyrophosphate and geranylgeranylpyrophosphate, which are precursors of cholesterol biosynthesis.[23] These intermediates serve as important lipid attachments for the posttranslational modification of proteins, such as nuclear lamins, Ras, Rho, Rac and Rap. Protein isoprenylation permits the covalent attachment, subcellular localization and intracellular trafficking of membrane-associated proteins.[23] Given that isoprenylated proteins might control diverse cellular functions, it was not surprising that statins might have additional beneficial effects on EPCs beyond lipid lowering.

Statins and EPC Mobilization

One effect of statin therapy seems to be increased mobilization of EPCs. For example, a rodent study demonstrated that initiation of statin therapy (simvastatin, 1 mg/kg) led to a 2.4-fold increase of EPCs after 2 weeks and was sustained through 4 weeks of statin treatment (2.5-fold increase).[24] In addition, in a clinical study, 20 patients with chronic heart failure randomized to 4 weeks of simvastatin (10 mg/d) or ezetimibe (10 mg/d) treatment demonstrated that simvastatin treatment increased the number of functionally active EPCs, an effect that was not observed after ezetimibe therapy.[25] Despite these compelling findings, neither report identified a putative mechanism. Another study clearly illustrated that increased endothelial nitric oxide (eNO) availability was required for statin-induced improvement of EPC mobilization, offering some evidence of a mechanism. The statin-dependent changes in EPC mobilization were only observed in wild-type and not eNO synthase $(eNOS)^{-/-}$ mice.[26] An additional report further elaborated on the statin-dependent mechanism by demonstrating that atorvastatin, administered 10 mg/d for 12 months, decreased miR-221 and miR-222 levels. Both miR-221 and miR-222 inhibited cell migration, tube formation and wound healing in endothelial cells (ECs) *in vitro* and were negatively related to the number of EPCs in CAD patients.[27] It is likely that the earlier described 2 mechanisms are