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Spotlight on endothelial progenitor cell inhibitors: short review

Thomas Thum and Johann Bauersachs

Abstract: Endothelial progenitor cells (EPCs) are bone-marrow-derived cells that enter the systemic circulation to replace defective or injured mature endothelial cells. EPCs also contribute to neovascularization and limit the progression of atherosclerosis. Patients with reduced EPC levels or dysfunctional EPCs are at increased risk for coronary artery disease. Drug-mediated improvement of the mobilization, differentiation, function and homing of EPCs to sites of ischemia or injured endothelium may therefore be a promising novel therapeutic approach for various cardiovascular diseases. On the other hand, endogenous inhibitors of EPCs could also be valuable drug targets. The identification of EPC inhibitors and the development of novel drugs that can efficiently regulate production or elimination of these molecules may also be a promising approach for the future treatment of atherosclerosis. In the present review we summarize potential endogenous and exogenous inhibitors of EPCs, such as oxidized low-density lipoproteins, angiotensin II, glucose, cigarette smoke and others. Whenever possible, we also describe the underlying molecular events. Drug-induced mobilization and improvement of EPC function, as well as reduction of EPC inhibitors, is likely to enhance endothelial function and reduce atherosclerotic processes.

Key words: drug therapy; endothelial progenitor cells; endothelium; nitric oxide; reactive oxygen species

Introduction

Endothelial progenitor cells (EPCs) are bone-marrow-derived cells that may enter the systemic circulation to replace diseased mature endothelial cells in blood vessels.[1] Endothelial cell injury is the main stimulus for the development of atherosclerotic plaques.[2] EPCs contribute significantly to endothelial function and may represent a circulating repair pool of endothelial cells.[3–5] Suppression of this pool is considered to have detrimental effects on the cardiovascular system. Patients with reduced numbers of EPCs are at increased risk for endothelial injury and, in general, for atherosclerotic plaque formation.[4] Moreover, inadequate coronary collateral development in patients with coronary heart disease is associated with reduced numbers of circulating EPCs.[6] Both endothelial function and damage appear to be the result of an altered balance between endothelial injury and the capacity for repair (Figure 1). In a mathematical model it was demonstrated that only minor elevations in the amount of circulating EPCs could significantly delay endothelial layer defects over time.[7] This is, however, only a theoretical model and does not account for EPC functional capacity, which, besides the number of EPCs, appears to be a major determinant of EPC-mediated beneficial effects.[8] In general, it is assumed that increasing the amount *and* enhancing the function of EPCs will improve cardiovascular outcome.

Thus, much attention has been given to drugs that enhance the peripheral EPC pool, such as granulocyte-macropage colony-stimulating factor,[9] various HMG-CoA (3-hydroxyl-3-methylglutaryl coenzyme A) reductase inhibitors,[10,11] erythropoietin[12] and, recently, angiotension-converting enzyme (ACE) inhibitors.[13] Drug-mediated improvement of EPC mobilization and function was shown to limit atherosclerosis and restenotic processes in coronary and carotid arteries, and to enhance re-endothelialization and neovascularization after myocardial infarction, although long-term effects need to be identified.[14–16] Moreover, recent research is additionally strongly focused on the transplantation of progenitor cells[17,18] and, although early results are convincing, long-term outcomes and potential adverse side effects of this invasive procedure are unclear.[19]

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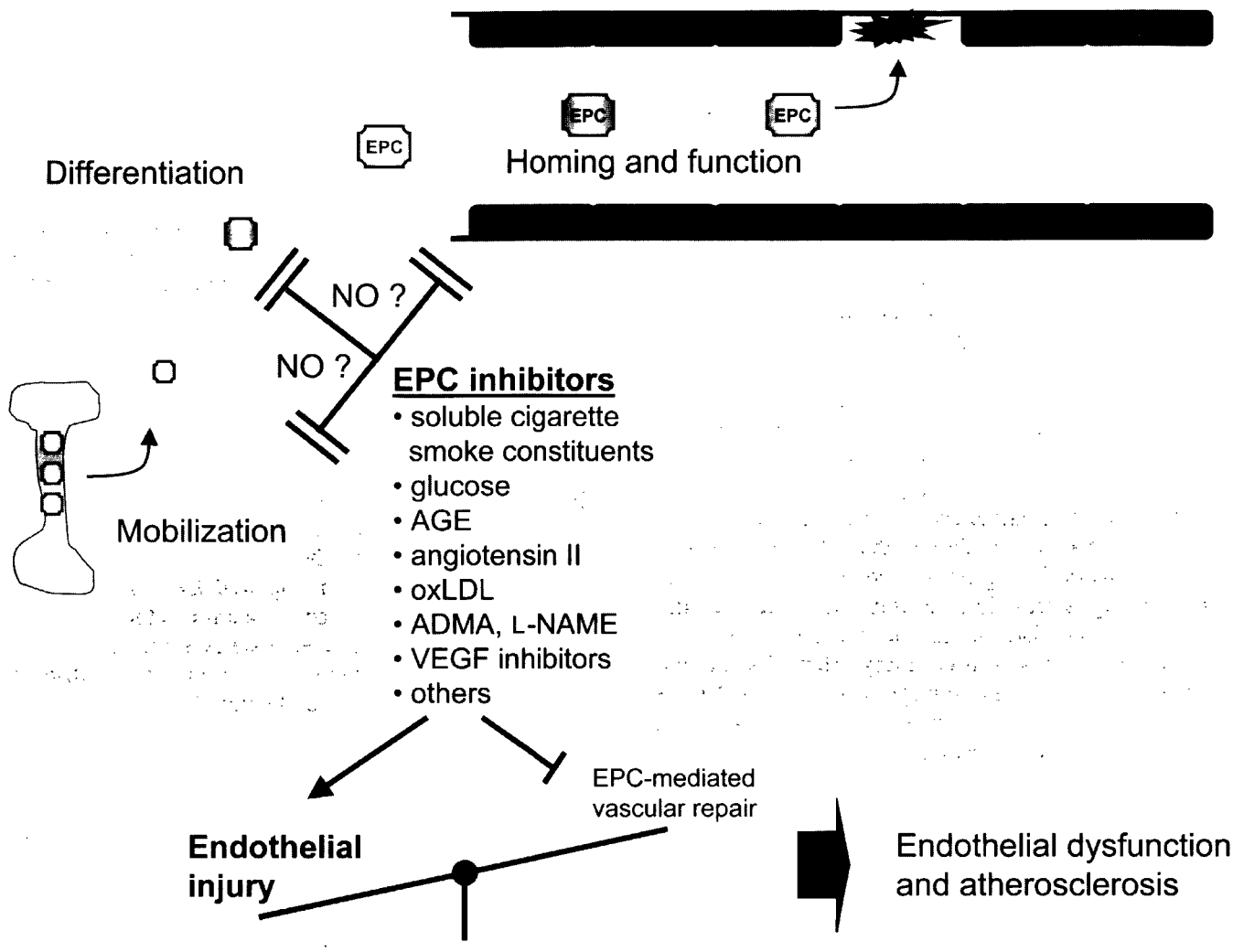

Figure 1 Schematic overview of the potential role of EPC inhibitors on the mobilization, differentiation, homing and function of EPCs. Impairment of EPC-mediated vascular repair and direct toxic endothelial effects are likely to contribute to the development of endothelial dysfunction and atherosclerosis. ADMA, asymmetric dimethylarginine; AGE, advanced glycation end-products; EPC, endothelial progenitor cell; L-NAME, *N*(*G*)-nitro-L-arginine methyl ester; NO, nitric oxide; oxLDL, oxidized low-density lipoprotein; VEGF, vascular endothelial growth factor.

There is uncertainty about potential inhibitors of EPCs, although their therapeutic reduction could be an interesting way of enhancing the beneficial effects of EPCs. Understanding the molecular mechanisms of EPC mobilization, differentiation, homing, adhesion and function is a first step to developing useful drugs that may normalize disturbed EPC biology.

Endogenous inhibitors of EPCs

In general, the number of EPCs is tightly correlated with cardiac risk factors and the Framingham risk score.[3,15] Certain endogenous mediators of vascular dysfunction and injury may not only adversely affect the mature endothelium of the vessel wall but diminish the number of circulating progenitor cells.

Nitric oxide inhibitors and reactive oxygen species

One of the most important functions of endothelial cells is the production of nitric oxide (NO) by the endothelial NO synthase (eNOS).[20] Besides its well-known protective impact on vascular tone and wall homeostasis, NO is essentially involved in the mobilization and function of vascular progenitor cells. Indeed, Dimmler's group was the first to demonstrate that eNOS knockout mice suffer from decreased EPC mobilization.[21] Moreover, a variety of EPC mobilizing agents such as statins act, at least in part, via NO-mediated effects.[14] Our group recently demonstrated that bone marrow-derived NO is correlated with enhanced EPC mobilization to the circulation after myocardial infarction (T Thum, D Fraccarollo, P Galuppo, T Tsikas, G Ertl, J Bauersachs, unpublished

manuscript). Particular attention should therefore be given to potential endogenous inhibitors of NO availability that may adversely affect EPC mobilization and/or function. One interesting candidate could be the endogenous NO synthase inhibitor asymmetric dimethylarginine (ADMA), which induces endothelial dysfunction[22] and impairs angiogenesis in vivo.[23] Furthermore, ADMA levels are closely correlated with coronary artery disease and acute coronary events.[24,25] Preliminary data suggest that ADMA plasma levels are inversely correlated with circulating progenitor cells.[26] The effects of ADMA on EPCs are currently under intense investigation. The treatment of cells with ADMA leads to increased intracellular production of reactive oxygen species (ROS).[27] ROS, in turn, may hamper the function of EPCs, although EPCs from healthy controls are equipped with anti-oxidative enzymes such as manganese superoxide dismutase.[28] Recently, it has been shown that the endogenous release of ROS is higher in the EPCs of patients with coronary artery disease compared with healthy controls.[29] Likewise, antioxidative enzymes were repressed in patients with coronary artery disease and showed markedly repressed migratory capacity.[29] ROS should therefore be considered as an important endogenous inhibitor of EPCs.[26]

Oxidized lipoproteins

The direct toxic effects of oxidized low-density lipoproteins (oxLDL) on endothelium are well known.[30,31] Recent evidence also suggests adverse effects of oxLDL on EPCs.[32] Treatment of cultured peripheral blood mononuclear cells with oxLDL reduces their differentiation into EPCs.[32] Likewise, proliferation, migration and in vitro vasculogenesis were reduced in a concentration-dependent manner, demonstrating negative quantitative and qualitative effects on EPCs.[33] Direct toxic effects of oxLDL include exaggerated production of ROS, enhanced expression of adhesion molecules, and downregulation of eNOS, leading to endothelial dysfunction.[30,31] Indirectly, by impairing EPC differentiation and function, oxLDL probably reduces the endothelial repair pool, contributing to its pro-atherogenic effects.

Glucose and diabetes mellitus

Chronically elevated glucose levels in diabetic patients correlate with endothelial dysfunction and atherosclerosis.[34,35] Tepper et al were the first to demonstrate that human EPCs from patients with type 2 diabetes exhibit impaired proliferation, adhesion and incorporation into vascular structures.[36] Likewise, Loomans and colleagues determined levels of circulating EPCs in type 1 diabetes.[37] They found that EPC numbers were reduced by 40–50%, which could explain the impaired wound angiogenesis and healing in diabetes, as well as reduced collateral formation in ischemia. Although these studies suggest increased glucose levels as an underlying cause, there is no conclusive evidence. Glucose-stimulated ROS production within EPCs may be involved. Indeed, high glucose levels stimulate ROS production via a protein kinase C-dependent activation of NADPH oxidase in endothelial cells.[38,39] Moreover, eNOS exposure to oxidants causes increased enzymatic uncoupling and generation of oxygen superoxides in diabetes.[40] In addition, some of the vasculotoxic effects are mediated by advanced glycation end-products.[41] Thus, more studies are required to determine the mechanism by which diabetes mellitus impairs EPC function.

Endostatin

Endostatin is an endogenous anti-angiogenic molecule that reduces the number and clonogenic potential of circulating EPCs.[42] Cellular stress upregulates endothelial endostatin release[43] and therefore enhanced endostatin levels may also result in impaired EPC mobilization and function, as well as progression of atherosclerosis in certain patients. Indeed, endostatin is enhanced in the pericardial fluid of patients with coronary artery disease and reduced formation of collaterals.[44]

C-reactive protein

As elevated levels of C-reactive protein (CRP) emerged as powerful predictors of cardiovascular disease,[45,46] their role in the biology of EPC may also be of importance. Indeed, human recombinant CRP directly inhibits EPC differentiation, survival, function, angiogenesis and the response to chronic ischemia.[47] These effects were attributed to a reduction of eNOS expression in EPCs and could be prevented by pretreatment of EPCs with the peroxisome proliferator-activated receptor-gamma agonist rosiglitazone.[47] Moreover, CRP additionally decreases secretion of arteriogenic chemokines in EPCs and may impair their vascular regenerative capacity.[48] Thus, elevated levels of CRP in patients with coronary artery disease[46] could be mechanistically correlated with diminished EPC function in such patients, as reported previously.[8] It is important to note that statins are able to reduce CRP levels,[49] which may contribute to their well-known EPC-inducing properties.[11]

Angiotensin II

Angiotensin II is the effector peptide of the renin-angiotensin system and has been implicated in the pathogenesis of atherosclerosis and coronary artery disease.[50] Angiotensin II potentiates vascular endothelial growth factor (VEGF)-induced human EPC proliferation and network formation through upregulation of the VEGF receptor kinase domain-containing receptor after 24 hours of treatment.[51] However, after longer periods (>7 days), angiotensin II has a significant inhibitory effect on the mitogenic activity of EPCs.[52]

Moreover, angiotensin II accelerates the rate of senescence by diminishing telomerase activity in EPCs.[53] Thus, increased angiotensin II levels in various cardiovascular diseases may increase atherosclerotic processes by enhanced senescence of circulating EPCs. Moreover, it has been reported that the treatment of patients with stable coronary artery disease with ACE inhibitors resulted in an increase of circulating EPCs.[13] Furthermore, in rats with myocardial infarction, we were able to demonstrate that ACE inhibition resulted in an improved matrix metalloproteinase 9-mediated mobilization of EPCs from bone marrow to the circulation (T Thum, D Fraccarollo, P Galuppo, T Tsikas, G Ertl, J Bauersachs, unpublished manuscript).

Exogenous inhibitors of EPCs

Pharmaceutical drugs or other xenobiotics that reduce the circulating EPC pool or cause EPC dysfunction can theoretically impair endothelial function and promote atherosclerosis.

Although not investigated in this context so far, inhibitors of the VEGF protein could be such candidates. VEGF inhibitors were originally designed for anti-angiogenic cancer treatment and indeed early results are promising.[54] However, VEGF also plays a role in the NO-mediated mobilization of progenitor cells[10,14] (and T Thum, D Fraccarollo, P Galuppo, T Tsikas, G Ertl, J Bauersachs, unpublished manuscript) and systemic inhibition may lead to impaired endothelial repair mechanisms.

Moreover, exogenous substances that affect the bioavailability of NO may additionally affect EPCs. Indeed, infusion with the NO synthase inhibitor *N*-nitro-L-arginine methyl ester or with ADMA resulted in a decrease of effective renal plasma flow, and an increase in renovascular resistance and blood pressure.[55] It is likely that this treatment effectively reduces the eNOS activity of blood vessels and bone marrow, thus also leading to a reduction in EPC mobilization and function.

In addition, cigarette smoke was identified as an exogenous inhibitor of EPCs.[56] Indeed, the number of circulating EPCs was reduced in chronic smokers and smoking cessation led to a rapid restoration of EPC levels.[57] This may be explained in part by the fact that smoking inhibits the release of physiological amounts of NO, which is important for EPC mobilization.[21] Nicotine was found to increase the number and function of EPCs in vitro,[57] as well as angiogenesis,[58] so the responsible cigarette smoke constituents causing impairment of EPCs are still not known and need to be identified.

Conclusion

Endogenous and exogenous inhibitors of EPCs are likely to contribute to endothelial dysfunction and atherosclerosis progression by impairment of the self-renewal capacity of endothelium. The identification of such substances is therefore of clinical importance. Therapeutic reduction of EPC inhibitors may be a promising approach for the future treatment of atherosclerosis.

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