



HAL
open science

Spotlight on endothelial progenitor cell inhibitors: short review

Thomas Thum, Johann Bauersachs

► **To cite this version:**

Thomas Thum, Johann Bauersachs. Spotlight on endothelial progenitor cell inhibitors: short review. Vascular Medicine, 2005, 10 (1), pp.S59-S64. 10.1177/1358836X0501000109 . hal-00571348

HAL Id: hal-00571348

<https://hal.science/hal-00571348>

Submitted on 1 Mar 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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-macrophage colony-stimulating factor,⁹ various HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase inhibitors,^{10,11} erythropoietin¹² and, recently, angiotensin-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

Medizinische Klinik, Kardiologie, Julius-Maximilians-Universität Würzburg, Würzburg, Germany

Address for correspondence: Thomas Thum, Bayrische Julius-Maximilians-Universität, Universitätsklinikum, Medizinische Klinik I, Kardiologie, Joseph-Schneider Str. 2, 97080 Würzburg, Germany. Tel: +49 931 201 36154; Fax: +49 931 201 36953; E-mail: Thum_t@klinik.uni-wuerzburg.de

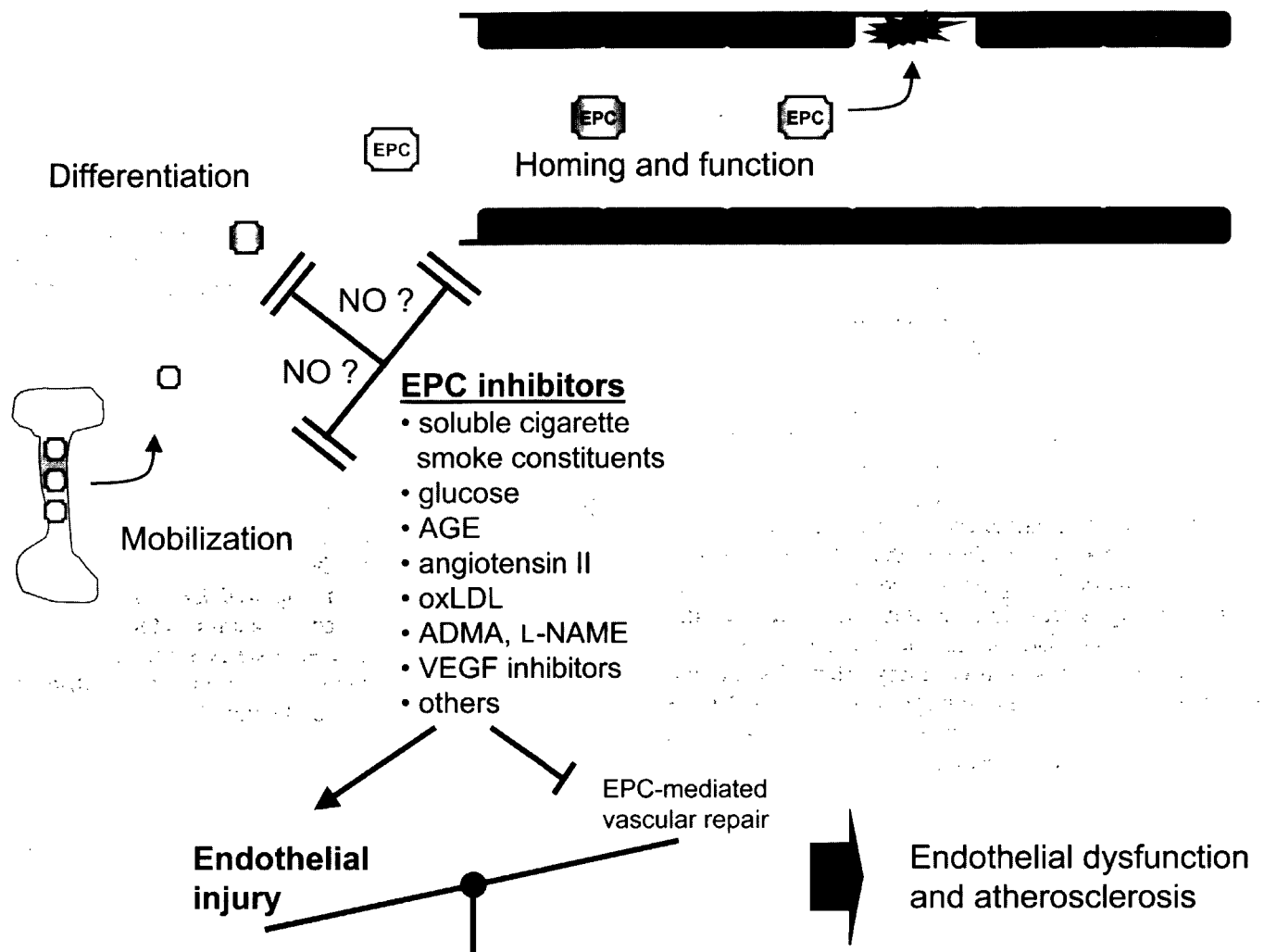


Figure 1 Schematic overview of the potential role of EPC inhibitors on the mobilization, differentiation, homing and function of EPCs. Impairment of EPC-mediated vascular repair and direct toxic endothelial effects are likely to contribute to the development of endothelial dysfunction and atherosclerosis. 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 dysfunction²² 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 release⁴³ 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 disease⁴⁶ 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.

Acknowledgment

This work was supported in part by the Ernst und Berta Grimmke-Stiftung (TT).

References

- Asahara T, Murohara T, Sullivan A et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997; **275**: 964–67.
- Ross R. Atherosclerosis – an inflammatory disease. *N Engl J Med* 1999; **340**: 115–25.
- Cho HJ, Kim HS, Lee MM et al. Mobilized endothelial progenitor cells by granulocyte-macrophage colony-stimulating factor accelerate reendothelialization and reduce vascular inflammation after intravascular radiation. *Circulation* 2003; **108**: 2918–25.
- Hill JM, Zalos G, Halcox JP et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 2003; **348**: 593–600.
- Isner JM, Kalka C, Kawamoto A, Asahara T. Bone marrow as a source of endothelial cells for natural and iatrogenic vascular repair. *Ann N Y Acad Sci* 2001; **953**: 75–84.
- Lambiase PD, Edwards RJ, Anthopoulos P et al. Circulating humoral factors and endothelial progenitor cells in patients with differing coronary collateral support. *Circulation* 2004; **109**: 2986–92.
- Op Den Buijs J, Musters M, Verrips T, Post JA, Braam B, Van Riel N. Mathematical modeling of vascular endothelial layer maintenance: the role of endothelial cell division, progenitor cell homing and telomere shortening. *Am J Physiol Heart Circ Physiol* 2004; **287**: 2651–58.
- Heeschen C, Lehmann R, Honold J et al. Profoundly reduced neovascularization capacity of bone marrow mononuclear cells derived from patients with chronic ischemic heart disease. *Circulation* 2004; **109**: 1615–22.
- Takahashi T, Kalka C, Masuda H et al. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat Med* 1999; **5**: 434–38.
- Dimmeler S, Aicher A, Vasa M et al. HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. *J Clin Invest* 2001; **108**: 391–97.
- Vasa M, Fichtlscherer S, Adler K et al. Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease. *Circulation* 2001; **103**: 2885–90.
- Bahlmann FH, De Groot K, Spandau JM et al. Erythropoietin regulates endothelial progenitor cells. *Blood* 2004; **103**: 921–26.
- Min TQ, Zhu CJ, Xiang WX, Hui ZJ, Peng SY. Improvement in endothelial progenitor cells from peripheral blood by ramipril therapy in patients with stable coronary artery disease. *Cardiovasc Drugs Ther* 2004; **18**: 203–209.

- 14 Landmesser U, Engberding N, Bahlmann FH et al. Statin-induced improvement of endothelial progenitor cell mobilization, myocardial neovascularization, left ventricular function, and survival after experimental myocardial infarction requires endothelial nitric oxide synthase. *Circulation* 2004; **110**: 1933–39.
- 15 Vasa M, Fichtlscherer S, Aicher A et al. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 2001; **89**: 1–7.
- 16 Kong D, Melo LG, Gnecci M et al. Cytokine-induced mobilization of endothelial progenitor cells enhances repair of injured arteries. *Circulation* 2004; **111**: 2039–46.
- 17 Wollert KC, Meyer GP, Lotz J et al. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet* 2004; **364**: 141–48.
- 18 Schachinger V, Assmus B, Britten MB et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOP-CARE-AMI Trial. *J Am Coll Cardiol* 2004; **44**: 1690–99.
- 19 Yoon YS, Park JS, Tkebuchava T, Luedeman C, Losordo DW. Unexpected severe calcification after transplantation of bone marrow cells in acute myocardial infarction. *Circulation* 2004; **109**: 3154–57.
- 20 Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; **288**: 373–76.
- 21 Aicher A, Heeschen C, Mildner-Rihm C et al. Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. *Nat Med* 2003; **9**: 1370–76.
- 22 Boger RH, Sydow K, Borlak J et al. LDL cholesterol upregulates synthesis of asymmetric dimethylarginine in human endothelial cells: involvement of S-adenosylmethionine-dependent methyltransferases. *Circ Res* 2000; **87**: 99–105.
- 23 Jang JJ, Ho H-KV, Kwan HH, Fajardo LF, Cooke JP. Angiogenesis is impaired by hypercholesterolemia – role of asymmetric dimethylarginine. *Circulation* 2000; **102**: 1414–19.
- 24 Boger RH. Asymmetrical methylarginine (ADMA) as a cardiovascular risk factor: epidemiological and prospective data. *Dtsch Med Wochenschr* 2004; **129**: 820–24.
- 25 Valkonen VP, Paiva H, Salonen JT et al. Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. *Lancet* 2001; **358**: 2127–28.
- 26 Thum T, Tsikas T, Stein S et al. Suppression of endothelial progenitor cells in human coronary artery disease by the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine. *J Am Coll Cardiol* 2005 (in press).
- 27 Scalera F, Borlak J, Beckmann B et al. Endogenous nitric oxide synthase inhibitor asymmetric dimethyl L-arginine accelerates endothelial cell senescence. *Arterioscler Thromb Vasc Biol* 2004; **24**: 1816–22.
- 28 Dernbach E, Urbich C, Brandes RP, Hofmann WK, Zeiher AM, Dimmeler S. Anti-oxidative stress-associated genes in circulating progenitor cells: evidence for enhanced resistance against oxidative stress. *Blood* 2004; **104**: 3591–97.
- 29 Fischer-Rasokat U, Dernbach E, Urbich C. Increased endogenous reactive oxygen species generation in endothelial progenitor cells of patients with coronary artery disease are associated with functional impairment. Proceedings of the Scientific Sessions Meeting, American Heart Association; 2004; New Orleans. *Circulation* 2004; **110**(suppl): abstract 205.
- 30 Li D, Mehta JL. Antisense to LOX-1 inhibits oxidized LDL-mediated upregulation of monocyte chemoattractant protein-1 and monocyte adhesion to human coronary artery endothelial cells. *Circulation* 2000; **101**: 2889–95.
- 31 Thum T, Borlak J. Mechanistic role of cytochrome P450 monooxygenases in oxidized low-density lipoprotein-induced vascular injury – therapy through LOX-1 receptor antagonism? *Circ Res* 2004; **94**: 1–13.
- 32 Imanishi T, Hano T, Matsuo Y, Nishio I. Oxidized low-density lipoprotein inhibits vascular endothelial growth factor-induced endothelial progenitor cell differentiation. *Clin Exp Pharmacol Physiol* 2003; **30**: 665–70.
- 33 Imanishi T, Hano T, Sawamura T, Nishio I. Oxidized low-density lipoprotein induces endothelial progenitor cell senescence, leading to cellular dysfunction. *Clin Exp Pharmacol Physiol* 2004; **31**: 407–13.
- 34 Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 1998; **339**: 229–34.
- 35 Hurst RT, Lee RW. Increased incidence of coronary atherosclerosis in type 2 diabetes mellitus: mechanisms and management. *Ann Intern Med* 2003; **139**: 824–34.
- 36 Tepper OM, Galino RD, Capla JM et al. Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. *Circulation* 2002; **106**: 2781–86.
- 37 Loomans CJ, de Koning EJ, Staal FJ et al. Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes. *Diabetes* 2004; **53**: 195–99.
- 38 Inoguchi T, Sonta T, Tsubouchi H et al. Protein kinase C-dependent increase in reactive oxygen species (ROS) production in vascular tissues of diabetes: role of vascular NAD(P)H oxidase [Review]. *J Am Soc Nephrol* 2003; **14** (8 suppl 3): S227–32.
- 39 Hink U, Li H, Mollnau H et al. Mechanisms underlying endothelial dysfunction in diabetes mellitus. *Circ Res* 2001; **88**: 14–22.
- 40 Zou MH, Shi C, Cohen RA. Oxidation of the zinc-thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite. *J Clin Invest* 2002; **109**: 817–26.
- 41 Xu B, Chibber R, Ruggiero D et al. Impairment of vascular endothelial nitric oxide synthase activity by advanced glycation end products. *FASEB J* 2003; **17**: 1289–91.
- 42 Capillo M, Mancuso P, Gobbi A et al. Continuous infusion of endostatin inhibits differentiation, mobilization, and clonogenic potential of endothelial cell progenitors. *Clin Cancer Res* 2003; **9**: 377–82.
- 43 Deininger MH, Wybraniec WA, Graepler FT, Lauer UM, Meyermann R, Schluesener HJ. Endothelial endostatin release is induced by general cell stress and modulated by the nitric oxide/cGMP pathway. *FASEB J* 2003; **17**: 1267–76.
- 44 Panchal VR, Rehman J, Nguyen AT et al. Reduced pericardial levels of endostatin correlate with collateral development in patients with ischemic heart disease. *J Am Coll Cardiol* 2004; **43**: 1383–87.
- 45 Willerson JT, Ridker PM. Inflammation as a cardiovascular risk factor. *Circulation* 2004; **109**: 2–10.
- 46 Ridker PM, Rifai N, Rose L et al. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med* 2002; **347**: 1557–65.

- 47 Verma S, Kuliszewski MA, Li SH et al. C-reactive protein attenuates endothelial progenitor cell survival, differentiation, and function: further evidence of a mechanistic link between C-reactive protein and cardiovascular disease. *Circulation* 2004; **109**: 2058–67.
- 48 Suh W, Kim KL, Choi JH et al. C-reactive protein impairs angiogenic functions and decreases the secretion of arteriogenic chemo-cytokines in human endothelial progenitor cells. *Biochem Biophys Res Commun* 2004; **321**: 65–71.
- 49 Albert MA, Danielson E, Rifai N et al. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. *JAMA* 2001; **286**: 64–70.
- 50 Schmidt-Ott KM, Kagiya S, Phillips MI. The multiple actions of angiotensin II in atherosclerosis. *Regul Pept* 2000; **93**: 65–77.
- 51 Imanishi T, Hano T, Nishio I. Angiotensin II potentiates vascular endothelial growth factor-induced proliferation and network formation of endothelial progenitor cells. *Hypertens Res* 2004; **27**: 101–108.
- 52 Imanishi T, Hano T, Takarada S, Nishio I. Biphasic effects of angiotensin II on the proliferative activity in endothelial progenitor cells. Proceedings of the Scientific Sessions Meeting, American Heart Association, 2004, New Orleans. *Circulation* 2004; **110**(suppl): abstract 717.
- 53 Ferrara N, Alitalo K. Clinical applications of angiogenic growth factors and their inhibitors. *Nat Med* 1999; **5**: 1359–64.
- 54 Ziche M, Donnini S, Morbidelli L. Development of new drugs in angiogenesis. *Curr Drug Targets* 2004; **5**: 485–93.
- 55 Kielstein JT, Impraim B, Simmel S et al. Cardiovascular effects of systemic nitric oxide synthase inhibition with asymmetrical dimethylarginine in humans. *Circulation* 2004; **109**: 172–77.
- 56 Kondo T, Hayashi M, Takeshita K et al. Smoking cessation rapidly increases circulating progenitor cells in peripheral blood in chronic smokers. *Arterioscler Thromb Vasc Biol* 2004; **24**: 1442–47.
- 57 Wang X, Zhu J, Chen J, Shang Y. Effects of nicotine on the number and activity of circulating endothelial progenitor cells. *J Clin Pharmacol* 2004; **44**: 881–89.
- 58 Heeschen C, Jang JJ, Weis M et al. Nicotine stimulates angiogenesis and promotes tumor growth and atherosclerosis. *Nat Med* 2001; **7**: 833–39.