

Human endothelial progenitor cells, endothelial dysfunction, hypertension and ACE inhibitors

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ABSTRACT. During the last decades, the role of the vascular endothelium has been well documented and investigated and all the studies that were made lead to the conclusion that this vascular endothelium isn't just a monolayer and a simple barrier, but a complex organ with various functions. This monolayer provides a "first line" physiological defense against atherosclerosis and it is considered to play an important role in inflammation and angiogenesis.

The mature endothelial cells are involved in the repairment of the monolayer injury, thus having a limited capacity of recovery, thing that has lead to the investigation of the circulating endothelial progenitor cells and their role in this vascular healing.

Recent studies showed that these cells have a huge role in the fast endothelization of the areas touched by the vascular damage and that several cardiovascular risk factors, but also several cardiovascular diseases decrease the number and function of these helpful and important cells.

The treatment with ACE inhibitors seems to normalize and reverse not only the high blood pressure but also the number of the endothelial progenitor cells, and by this also the endothelial dysfunction.

In this review, we summarize the most important aspects about endothelial progenitor cells, endothelial dysfunction, hypertension, ACE inhibitors and also what they have in common.

KEYWORDS: stem cells, endothelial progenitor cells, EPCs, endothelial dysfunction, hypertension, ACE inhibitors.

INTRODUCTION

For many species of animals, the development of the vascular system is essential, during the embryonic phase. In this process, the local mesodermal precursors will differentiate into vascular and endothelial cells (ECs) to form a primary vascular plexus, process that has been named vasculogenesis. [1,2]. This process, vasculogenesis, was believed to occur only in the embryonic development and not in postnatal life, until the description of circulating endothelial progenitors (CEPCs). [1]

The hemangioblast, blood islands and angioblasts

During mammalian development, the hemangioblast from the yolk sac, the initial site of hematopoiesis and blood vessel formation, can differentiate in hematopoietic cells (HCs) and endothelial cells (ECs), as it has been seen by Murray in 1934. [3,4]

The most powerful evidence to support the idea of a common progenitor was the study of differentiating embryonic stem cell cultures, where the "blast colonies" were giving rise to both lineages from transiently. [3, 5]

The blood islands are aggregation of mesodermal cells that colonize the presumptive yolk sac. The peripheral cells (angioblasts) become ECs, whereas central cells become HCs. Angioblasts are defined as cells with some, but not all, the markers characteristic of an EC. [3,6] Endothelial cells that are derived from the yolk sac can induce the differentiation of mature myeloid, erythroid and lymphoid cells and their progenitors. [3,7,8,9] SCL/Tal-1 and VEGFR-2 (vascular endothelial growth factor receptor – 2) are co expressed in isolated mesodermal cells that give rise to ECs, while HCs seem to express only SCL/Tal-1. [3] The maturation of the blood vessel involves a sequential pattern in which SCL/Tal-1 and VEGFR-2 are expressed first, followed by PECAM-1, CD34, VE-cadherin and later Tie-2. The

expression of SCL/Tal-1 is finally down regulated by mature ECs. [3]

In 1963, Stumo et al. were the first to indicate the presence of circulating ECs using Darcon grafts. This study indicated that new endothelium on the flow surface of the grafts derived from blood-borne cells. [3] It has also been observed that circulating ECs are present in diseases marked by vascular injury as: sickle cell anemia, acute myocardial infarction, thrombotic thrombocytopenic purpura and active cytomegalovirus infection. [3, 10,11,12,13]

The total number of circulating ECs in normal adults is 2.6 ± 1.6 per ml of peripheral blood and at least half are microvascular as defined by CD34⁺. [3,13]

Specific/non specific markers of CEPCs

Immature EPCs and primitive HSCs (hematopoietic stem cells) are impossible to be differentiated as they share the same common surface. EPCs don't have an exclusive marker and both EPCs and mature ECs express similar endothelial-specific markers like: VEGFR-2, Tie-1, Tie-2, VE-cadherin. [3, 14, 15, 16, 17] But further, to identify the differences is difficult and complicated because of the fact that HCs subsets express markers similar to those of ECs, such as CD34, PECAM, Tie-1, Tie-2, Eph and VEGR-1, transcription factors such as SCL/Tal-1 and AML1, von Willebrand factor (vWF) [3, 18,19]. In 1997, Asahara et al., used a poly clonal antibody to the intracellular domain of VEGFR-2 to show that CD34⁺, VEGFR-2⁺ circulating EPCs form colonies that take up acetylated LDL. They injected into mice, rats and rabbits who were undergoing neovascularization because of hind limb ischemia, CD34⁺, VEGFR-2⁺, CD34⁺, VEGFR-2⁻ and observed that only CD34⁺ and VEGFR-2⁺ incorporate into the vasculature in a manner consistent with their being ECs. [3, 20]

Another hematopoietic stem cell marker, AC133⁺ is also expressed on EPCs subsets, but not mature ECs. [21]. AC133⁺ cells from granulocyte stimulating factor – mobilized peripheral blood can differentiate into ECs when cultured in the presence of VEGF and stem cell growth factor. [22]

AC133 has been shown to be the best selective marker for identifying EPC and that circulating CD34⁺, VEGFR-2⁺ and AC133⁺ cells constitute a phenotypically and functionally distinct population of circulating ECs that may play a role in postnatal vasculogenesis. [3]

The source of CEPCs

Most circulating EPCs reside in the bone marrow in close association with hematopoietic stem cells and the stroma. The local proliferation and transmigration of EPCs across the bone marrow/blood barrier may be promoted by these stem cells and stroma cells. [3] In adults, EPCs mobilized from bone marrow, migrate first from a quiescent niche within the bone

marrow into a permissive, proliferative microenvironment called vascular zone of marrow. [3] The MMP-9 (matrix metalloproteinase-9) as well as the survival/mitogenic activity of the stem cell cytokine SkitL appears critical during this process.[3] The release of EPCs from bone marrow into the circulation can be induced by granulocyte macrophage colony stimulating factor (GM-CSF) or VEGF and its critically dependent on the activity of endothelial nitric oxide synthase (eNOS) expressed by stromal cells in bone marrow. [3, 23] The EPCs in the peripheral blood may derive from the bone marrow and be not yet incorporated into the vessel wall. [3] Peripheral blood mononuclear cells from adult humans can be enriched in EPCs by addition of VEGF, FGF-2, insulin-like growth factor and EGF to the culture medium for 7-10 days. After local injection *in vivo*, these cells contribute to the formation of new vessels in the ischemic limb. [3,24]. Monocytic EPCs may enhance the angiogenic process via release of inflammatory mediator that stimulate granulation tissue formation. [3]

EPCs can be released into the circulation in response to angiogenic growth factors, chemokines and cytokines released following various stimuli, such as vascular trauma. [25,26] EPCs are believed to exert their function in two possible ways: by activating locally the ECs and/or by differentiating into adult endothelial cells that can be incorporated the damaged vessels. [25]

Endothelial dysfunction, endothelial progenitor cells and vascular repair

Atherosclerosis, a process poorly understood until now, despite intense efforts, is a progressive and complex pathology. It is characterized by the thickening of the arterial wall, with complications in various vascular beds. [25] It has been demonstrated by several studies that the endothelium, a thin layer of cells localized between the blood flow and the vascular wall, plays a very important role in the regulation of vascular tone and structure, and that this monolayer isn't a simple barrier, but a complex organ. [25] This complex organ, with paracrine and autocrine function, provides “a first line” physiological defense against atherosclerosis. The endothelium is now considered to be of a huge importance in maintaining the vascular homeostasis, in regulating the vascular structure and tone and to play an important key role in inflammation and angiogenesis. [25]

The dysfunction of this monolayer, of the endothelium, the so called endothelium dysfunction, is one of the earliest process that occur in the pathogenesis of atherosclerosis. The mechanisms that can contribute to this endothelial dysfunction are: oxidative stress, up regulation of adhesion molecules, augment in response of the inflammatory process and prothrombotic state. Also, the vasoactive peptides (such as: angiotensin II, endothelin-1) but also hypercholesterolemia,

hyperhomocysteinemia, hyperglycemia play an important role in this process, as well as the apoptosis of the endothelial cells. [25,27]

Oxidative stress can inhibit three major endothelium-dependent vasodilator pathways: nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factor. A reduction in the bioavailability of NO, the deterioration of prostanoid synthesis (including: prostacyclin, thromboxane A₂ and isoprostanes) and an increased release of endothelin-1 can contribute to this endothelial dysfunction. Also, the activation of peroxisome proliferator-activated receptors (PPARs) (a pathway of prostacyclin signaling) seem to have beneficial and important roles over the endothelium. This vasodilator peptide derived from endothelium, prostacyclin, is thought to have angiogenic properties that are closely linked to its specific action on the PPAR signaling pathway. [25,28,29]

A pivotal endothelium-derived substance, the nitric oxide (NO), a substance synthesized from the substance L-arginine via eNOS (endothelial NO synthase), have a major role in vasorelaxation, inhibition of leukocyte-endothelial adhesion, vascular smooth muscle cell proliferation and migration, and platelet aggregation. So, an alteration in NO production or in its activity is considered a major mechanism of endothelial dysfunction, thus contributing to atherosclerosis. [25,30] Although many studies had been conducted until now, studies which demonstrate the implications of this substance in the pathogenesis of atherosclerosis, no single mechanism can completely explain the endothelial dysfunction, and one explanation could be because of the fact that atherosclerosis is a complex disease process and that multiple regulatory mechanisms are involved in bioactivity of the endothelial NO. [25]

Recent studies showed that reduced EPCs levels seem to be correlated with endothelial dysfunction and with increased risk of cardiovascular disease, as well as various cardiovascular diseases and cardiovascular risk factors (hypertension, diabetes, hypercholesterolemia, smoking) are related to an impairment in EPCs, both in number and in function. [25,31,32] So, levels of circulating EPCs has been proposed as a surrogate index of cumulative cardiovascular risk. [25]

The quantification of EPCs can be done by two most used methods: (1) FACS analysis (fluorescence-activated cell sorting) of total blood cells or circulating mononuclear cells and (2) culture assay of blood-derived mononuclear cells. [25] The CEPCs are characterized by the expression of CD133, CD34 and VEGFR-2, so they can be identified and quantified based on the expression of these cell surface markers. [25,33] . The most used markers to identify human EPCs had been the marker combination of CD34⁺KDR⁺, CD34⁺CD133⁺ and CD34⁺CD133⁺ KDR⁺. [25,34] The EPCs are present in a very small number, especially in the circulatory blood of adults (aprox. 0.01% of all cells) [25].

Several studies had shown that the number of functionally active EPCs is influenced by various angiogenic cytokines, cardiovascular risk factors, by lifestyle modifications like: physical exercise, body weight loss, smoking cessations; and also by some pharmacological interventions. [25] Among risk factors, **hypertension** is the strongest predictor of EPCs migratory impairment. [35] In adult subjects without a history of cardiovascular disease, the number of circulating EPCs is inversely correlated with Framingham risk score, which includes systolic blood pressure as a major component. [35] Studies conducted both in hypertensive animals and humans demonstrated that one possible mechanism which can explain the low number of CEPCs in hypertensive patients is the accelerated senescence of EPCs. [35,36] Hypertension is associated with increased oxidative stress, an mechanism that affects both angiogenesis and vasculogenesis, so it may be that progenitor cell dysfunction plays a role in the pathogenesis of hypertension. [35,36] Increased ROS levels decrease bone marrow cell differentiation into cells of endothelial phenotype *in vitro* and hamper their therapeutic effect *in vivo*. [35]

Moreover, angiotensin II diminishes telomerase activity in EPCs, accelerates the onset of EPCs senescence through an increase in oxidative stress and affects *in vitro* EPC proliferation. [35]

In experimental models of hypertension, but also in patients with essential hypertension, because of the impaired endothelial dysfunction, there is a reduced vessel regeneration action in hypoxic underperfused tissues. [35] Several experimental and clinical studies have suggested that statins, ACE inhibitors (angiotensin-converting enzyme), ARBs (angiotensin II type 1 receptor blockers), PPAR- γ agonists and erythropoietin might improve the microcirculatory network. [35] The mechanisms of these actions are not well defined, but can likely include activation of the PI3-kinase/Akt pathway and endothelial nitric oxide synthase, as well as inhibition of NAD(P)H oxidase activity of progenitor cells. [35,37]

ACE-inhibitors are the first class of blockers of the renin-angiotensin system, a class of drugs with not only anti-hypertensive effects, but also with pleiotropic effects. They are the most effective antihypertensive drug class in improving endothelial-dependent vasodilatation in large arteries of patients with essential hypertension. [35,38,39,40]

There is a growing evidence that suggests that one of the pleiotropic effects of ACE-inhibitors on the cardiovascular system involves the modulation of the number and function of EPCs activity and this seems to be a class effect, confirmed by several studies of different molecules of the class (enalapril, Ramipril, perindopril, quinapril). [25, 41]

Treatment with enalapril seems to increase CEPCs levels by stimulating the mobilization, rather than

the maturation. Enalapril augments the concentration of circulating SFD-1 α , but decreases its concentration in the bone marrow in response to ischemic stress, suggesting that the reduced binding of EPCs to SFD-1 α in bone marrow may help to the release and mobilization of EPCs after ACE-inhibitor treatment. [41]

Also, the treatment with Ramipril for 4 weeks increased EPCs levels, in patients with stable CAD. They observed that Ramipril increased NO levels and that because of the activation of bradykinin B2-receptor pathway, it may contribute on the functional improvement of EPCs, that was seen after the treatment with ramipril. [25, 42]

Also, it was observed that the addition of quinapril and metoprolol to EPC therapy induced the neovascularization and it reduced the number of apoptotic cardiomyocytes, thus without a completely understood mechanism. [25, 43]

A long-acting ACE-inhibitor, perindopril, has demonstrated to augment the number of CEPCs and to re-establish the ability of mononuclear cells from the bone marrow to differentiate into EPCs in a hind limb ischemia model in spontaneously hypertensive rats, alone or combined with indapamide. [25, 44]

CONCLUSIONS

As a conclusion, there is enough evidence that support the idea that ACE inhibition improves the biology of EPCs independently of a vasodilator or hemodynamic effect, although many questions have no answer involving the angiogenic effects of the drugs: the clear role of NO in the vasculogenesis induced by ACE inhibitors, is efficacy and potency the same for all the molecules of the class regarding their angiogenic action, the EPC improvement is the same in patients with other diseases like: metabolic syndrome, diabetes [25] ? Are ACE inhibitors the class of antihypertensive drugs with the best cardiovascular benefits ?

REFERENCES

1. Timmermans F., Plum J., Yoder M. C., Ingram D. A., Vandekerckhove B., Case J., Endothelial progenitor cells: identity defined?. *J. Cell. Mol. Med.* , Vol 13, No 1, 2009, pp 87-102
2. Carmeliet P. , Mechanisms of angiogenesis and arteriogenesis, *Nat Med*, 2000; 6:389-95
3. Ribatti D., Nico B., Crivellato E., Vacca A., Endothelial progenitor cells in health and disease, *Histol Histopathol*, 2005, 20: 1351-1358
4. Murray P.D.F, The development in vitro of blood of the chick embryo, *Proc. Royal Soc. B*, 1934, 111, 497 – 527
5. Choi K., Kennedy M., Kazarov A., Papadimitriou J.C. and Keller C., A common precursor for hematopoietic and endothelial cells, *Development*, 1998, 725 – 732
6. Risau W., Flamme I, Vasculogenesis, *Annu. Rev. Cell Biol*, 1995, 11, 73-91
7. Yoder M.C., Papaioannou V.E., Breitfeld P.P. and Williams D.A. , Murine yolk sac endoderm-and mesoderm-derived cell lines support in vitro growth and differentiation of hematopoietic cells, *Blood*, 1994, 83, 2436 – 2443.
8. Fennie C., Cheng J., Dowbenko D., Young P. and Lasky L.A., CD34⁺ endothelial cell lines derived from murine yolk sac induce the proliferation and differentiation of yolk sac CD34⁺ hematopoietic progenitors, *Blood*, 1995, 86, 4454 – 4467.
9. Lu L., Wang S. J. and Auerbach R., In vitro and in vivo differentiation into B cells, T cells and myeloid cells of primitive yolk sac hematopoietic precursor cells expanded >100-fold by coculture with a clonal yolk sac endothelial cell line, *Proc. Nat. Acad. Sci. USA*, 1996, 93, 14782 – 14787
10. Haldovec J., Prerovsky I., Stanek V. and Fabian J., Circulating endothelial cells in acute myocardial infarction and angina pectoris, *Klin. Wochenschr.* , 1978, 56, 1033-1036.
11. Grefte A., Van der Giessen M., Van Son W. and The T.H., Circulating cytomegalovirus (CMV)-infected endothelial cells in patients with an active CMV infection, *J. Infect. Dis.*, 1993, 167, 270-277.
12. Lefevre P., George F., Durand J.M. and Sampol J., Detection of circulating endothelial cells in thrombocytopenic purpura, *Thromb. Haemost.*, 1993, 69, 522 – 532
13. Solovey A., Li Y., Browne P., Chlong S., Wayner E., and Hebbel R.P., Circulating activated endothelial cell in sickle anemia, *New Engl. J. Med.*, 1997, 337, 1584 – 1590.
14. Sato T.N., Quin Y., Kozak C. A. and Audus K.L., Tie-1 and tie-2 define another class of putative receptor tyrosine kinase genes expressed in early embryonic vascular system, *Proc. Natl. Acad. Sci. USA*, 1993, 90, 9355-9358
15. Schnurch H. and Risau W., Expression of tie-2, a member of a novel family of receptor tyrosine kinases, in the endothelial cell lineage, *Development*, 1993, 119, 957 – 968
16. Vittet D., Prandini M.H., Berthier R., Schweitzer A., Martin – Sisteron H., Uzan G. and Dejana E., Embryonic stem cells differentiate in vitro to endothelial cells through successive maturation steps, *Blood*, 1996, 88, 3424-3431
17. Eichmann A., Corbel C., Nataf V., Breant C. and Le douarin N.M., Ligand-dependent development of the endothelial and hematopoietic lineages from embryonic mesodermal cells expressing vascular endothelial growth factor receptor-2, *Proc. Nat. Acad. Sci USA*, 1997, 5141 – 5146
18. Suda T., Takakura N. and Oike Y., Hematopoiesis and angiogenesis, *Int. J. Hematol.*, 2000, 71, 99 – 107
19. Lyden D. et al., Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells

- blocks tumor angiogenesis and growth, *Nat. Med.*, 2001, 7, 1194 – 1201
20. Asahara T. et al, Isolation of putative progenitor endothelial cells for angiogenesis, *Science*, 1997, 275, 965 – 967
 21. Yin A.H. et al., AC133, a novel marker for human hematopoietic stem and progenitor cells, *Blood*, 1997, 90, 5002 – 5012.
 22. Gehling U.M. et al, In vitro differentiation of endothelial cells from CD-133 positive progenitor cells, *Blood*, 2000, 3106 – 3112
 23. Aicher A. et al., Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells, *Nat. Med.*, 2003, 9, 1370 – 1376
 24. Kalka C. et al., Transplantation of *ex vivo* expanded endothelial progenitor cells for therapeutic neovascularization, *Proc. Nat. Acad. Sci. USA*, 2000, 97, 3422 – 3427
 25. Natalia Antonio, Rosa Fernandes et al., Stimulation of endothelial progenitor cells: a new putative effect of several cardiovascular drugs, *Eur. J. Clin. Pharmacol.*, 2010, 66:219 – 230
 26. Khoo C et al., Endothelial progenitor cells and their potential therapeutic applications, *Regen Med*, 2008, 3:863 – 876
 27. Endemann DH, Schiffrin EL, Endothelial dysfunction, *J Am Soc Nephrol*, 2004, 15:1983 – 1992
 28. Feletou M et al., Endothelial dysfunction: a multifaceted disorder (The Wiggers Award Lecture), *Am J Physiol Heart Circ Physiol*, 2006, 291:H985 – H1002
 29. Pola R et al., Comparative analysis of the *in vivo* angiogenic properties of stable prostacyclin analogs: a possible role for peroxisome proliferator-activated receptors, *J Mol Cell Cardiol*, 2008, 36:363-370
 30. Yang Z., Ming X-F, Recent advances in understanding endothelial dysfunction in atherosclerosis, *Clin Med Res*, 2006, 4:53-65
 31. Geft D. et al., Circulating endothelial progenitors cells in cardiovascular disorders, *Expert Rev Cardiovasc Ther*, 2008, 6:1115-1121
 32. Yamahara K et al., Potential use of endothelial progenitor cells for regeneration of the vasculature, *Ther Adv Cardiovasc Dis*, 2009, 3:17-27
 33. Hristov M, Weber C, Endothelial progenitor cells in vascular repair and remodeling, *Pharmacol Res*, 2008, 58:148-151
 34. Peichev M et al., Expression of VEGFR-2 and AC133 by circulating human CD34⁺ cells identifies a population of functional endothelial precursors, *Blood*, 2000, 85:952-958
 35. Jean Sebastien Silvestre, Bernard I. Levy, Circulating progenitor cells and cardiovascular outcomes: latest evidence on angiotensin-converting enzyme inhibitors, *Europ Heart J Supp*, 2009, 11, E17-E21
 36. Imanishi T. et al., Oxidized low-density lipoprotein induces endothelial progenitor cell senescence, leading to cellular dysfunction, *Clin Exp Pharmacol Physiol*, 2004, 31:407-413
 37. Besler C et al., Pharmacological approaches to improve endothelial repair mechanisms, *Expert Rev Cardiovasc Ther* 2008; 6:1071-1082
 38. Ghiadoni L et al., Ramipril dose-dependently increases nitric oxide availability in the radial artery of essential hypertension patients, *J Hypertens*, 2007, 25:361-366
 39. Anthony I et al., Angiotensin-converting enzyme inhibition restores flow-dependent and cold pressor test-induced dilatations in coronary arteries of hypertensive patients, *Circulation*, 1996, 94:3115-3122
 40. Ghiadoni L et al., Different effect of antihypertensive drugs on conduit artery endothelial function, *Hypertension*, 2003, 41:1281-1286
 41. Wang C. et al., Enalapril increases ischemia-induced endothelial progenitor cell mobilization through manipulation of the CD26 system, *J Mol Cell Cardiol*, 2006, 41:34-43
 42. Min TQ et al., 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
 43. Boyle AJ et al., Additive effects of endothelial progenitor cells combined with ACE inhibition and β -blockade on left ventricular function following acute myocardial infarction, *J Renin Angiotensin Aldosterone Syst*, 2005, 6:33-37
 44. You D. et al, Combination of the angiotensin-converting enzyme inhibitor perindopril and the diuretic indapamide activate postnatal vasculogenesis is spontaneously hypertensive rats, *J Pharmacol Exp Ther* 325:766-773

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