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REVIEW

Vascular dysfunction in diabetes: The endothelial progenitor cells as new therapeutic strategy

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Abstract

The vascular endothelium is a critical determinant of diabetes-associated vascular complications, and improving endothelial function is an important target for therapy. Diabetes mellitus contributes to endothelial cell injury and dysfunction. Endothelial progenitor cells (EPCs) play a critical role in maintaining endothelial function and might affect the progression of vascular disease. EPCs are essential to blood vessel formation, can differentiate into mature endothelial cells, and promote the repair of damaged endothelium. In diabetes, the circulating EPC count is low and their functionality is impaired. The mechanisms that underlie this reduced count and impaired functionality are poorly understood. Knowledge of the status of EPCs is critical for assessing the health of the vascular system, and interventions that increase the number of EPCs and restore their angiogenic activity in diabetes may prove to be particularly beneficial. The present review outlines current thinking on EPCs' therapeutic potential in endothelial dysfunction in diabetes, as well as evidence-based perspectives regarding their use for vascular regenerative medicine.

Key words: Diabetes mellitus; Vascular dysfunction; Endothelial progenitor cells

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VASCULAR FUNCTION AND DYSFUNCTION IN DIABETES

Diabetes is a metabolic disorder which is characterized by hyperglycemia and glucose intolerance due to insulin deficiency, impaired effectiveness of insulin action or, both.

Type 1 diabetes mellitus is caused by cellular-mediated autoimmune destruction of pancreatic islet beta cells, leading to loss of insulin production. It usually starts during childhood, but can occur at all ages. Type 2 diabetes mellitus accounts for 90%-95% of all diabetes and is more commonly found in people older than 45 who are overweight. There is strong evidence that genetics plays an important role as well. However, the prevalence of type 2 diabetes mellitus is increasing in children and young adults, mainly because of the higher rate of obesity in this population. Obesity, insulin resistance associated with diabetes, high cholesterol and high blood pressure form the most impor-

tant risk factors for cardiovascular disease (CVD). CVD is the major cause of death in people with type 2 diabetes mellitus[1].

The vascular manifestations associated with diabetes mellitus result from the dysfunction of several vascular physiological components, mainly involving the endothelium, vascular smooth muscle and platelets[2]. Over the last two decades it has become evident that the endothelium is not an inert, single-cell lining covering the internal surface of blood vessels, but in fact plays a crucial role in regulating vascular tone and structure. Importantly, a healthy endothelium inhibits platelet and leukocyte adhesion to the vascular surface and maintains a balance of prothrombic activity[3]. Hyperglycemia is the major causal factor in the development of endothelial dysfunction in diabetes mellitus. Although the mechanisms underlying this phenomenon are likely to be multifactorial, insulin resistance has been identified in several diseases that increase cardiovascular risk and mortality, such as diabetes, obesity, hypertension, metabolic syndrome, and heart failure[4].

In health, endothelial cell injury is mitigated by endogenous reparative processes. In diabetes sufferers, the imbalance in repair and injury results in micro-vascular changes, including apoptosis of micro-vascular cells, ultimately leading to diabetes-related complications.

Dysfunction of the endothelium in diabetes mellitus is characterized by changes in proliferation, barrier function, adhesion of other circulating cells, and sensitivity to apoptosis. Furthermore, it is suggested that diabetes mellitus modifies the angiogenic and synthetic properties of endothelial cells[5].

A variety of markers indicates endothelial dysfunction in diabetes mellitus, including poor EC-dependent vasodilation, increased blood levels of the von Willebrand factor (vWF), thrombomodulin, selectin, plasminogen activator inhibitor-1 (PAI-1), type Ⅳ collagen and tissue plasminogen activator (t-PA)[6]. Endothelial dysfunction is an early manifestation of vascular disease in type 2 diabetes patients but late in the course of those with type 1 diabetes[7]. Furthermore, studies have shown that the levels of vascular cell adhesion molecule 1 (VCAM-1) were more markedly elevated in type 1 diabetes patients with diabetic retinopathy, than in those patients with micro- or macroalbuminuria, whereas no difference in inter-cellular adhesion molecule 1 (ICAM-1) and endothelial-leukocyte adhesion molecule 1 (ELAM-1) levels was apparent in diabetes patients without diabetic retinopathy[8].

The loss of the endothelium modulator role may be a critical and initiating factor in the development of diabetic vascular disease. Endothelial dysfunction plays a key role in the pathogenesis of diabetic vascular disease. The endothelium controls the tone of underlying vascular smooth muscle through the production of vasodilator mediators. The endothelium-derived relaxing factors (EDRF) comprise nitric oxide (NO), prostacyclin, and a still-elusive endothelium-derived hyperpolarizing factor (EDHF). Impaired endothelium-dependent vasodilation has been demonstrated in various vascular beds of different animal models of diabetes, and in humans with type 1 and 2 diabetes[9-12]. Several other mechanisms of endothelial dysfunction have been reported, including impaired signal transduction or substrate availability, impaired release of EDRF, increased destruction of EDRF, enhanced release of endothelium-derived constricting factors, and decreased sensitivity of the vascular smooth muscle to EDRF. The principal mediators of hyperglycaemia-induced endothelial dysfunction may be activation of the protein kinase C, increased activity of the polyol pathway, and non-enzymatic glycation[13]. It is also known that hyperglycemia-induced oxidative stress plays a role in the development of vascular dysfunction[1]. In general, diabetic microvascular complications are typically associated with dysregulation of vascular remodeling and vascular growth with decreased responsiveness to ischemic/hypoxic stimuli and impaired or abnormal neovascularization[14].

Lack of endothelial regeneration and impaired angiogenesis contribute to the progression of diabetic micro- and macrovascular complications. Formation of stable vasculature in response to tissue injury is an essential event for the restoration of blood flow and the repair of affected tissue areas. Currently, clinical management of diabetic complications relies exclusively on pharmacological therapeutics that, in most cases, minimally affect the endothelial repair or regeneration, and, therefore these treatments have a modest influence on end organ dysfunction. Hence there is a need for therapeutic interventions that can accelerate the repair ofdysfunctional endothelium in the end organ, and restore blood flow, resulting in functional tissue generation. A promising novel therapeutic option for the replacement of damaged endothelial cells, i.e. re-endothelialization, as well as for the neovascularization of ischemic tissues, is the use of progenitor cells. In vascular biology, progenitor cells were first identified by Isner and Asahara in 1997, and they are known as endothelial progenitor cells (EPCs), able to initiate neovascularization[15].

EPCS: A BIOMARKER OF VASCULAR DYSFUNCTION IN DIABETES

The discovery of EPCs in human peripheral blood has advanced the field of cell-based therapeutics for many pathological conditions. It is known that EPCs could be released from bone marrow, fat tissue, vessel walls, especially adventitia, and possibly also from the spleen, the liver, and the intestine, into the blood, where they express CD133 at the early stage, then CD34/Flk-1, and also VEGFR2[16]. Experimental studies have shown that EPCs can be isolated from peripheral, umbilical cord, and bone marrow blood, and identified by specific markers, using flow cytometry. In most published studies, the amounts of circulating EPCs are determined by a culture[17]. EPCs are defined as fibronectin-adherent peripheral blood-derived cells uptaking acetilated low-density lipoprotein (LDL) and binding Ulex-selectin in culture, and then further characterized by the expression of surface markers. Peichev *et al*, showed that circulating CD34+CD133+KDR+

cells give rise to endothelial cells *in vitro*, and thus functionally correspond to the definition of EPCs. Therefore-three-fluorescence analysis of this cell subset may be another simple and elegant way to unambiguously identify and quantify circulating EPCs without culturing them[18]. At present there is no general agreement on methods for defining EPCs, and different studies have used different ways of identification and isolation.

It has been indicated that a strong correlation between cardiovascular risk factors and EPC number and function exists[19]. Diabetes mellitus has also been shown to adversely affect EPCs' number and function[20,21], and it has been suggested that a reduction in the number of EPCs might be useful as a surrogate marker of vascular dysfunction in diabetes[22]. As for the function of EPCs in diabetes, it has been shown that EPCs have decreased migratory ability, reduced proliferative capacity, and an altered cytokine/growth factor secretory profile. Changes in the function of EPCs decreases their repair mechanisms[14]. Consequently, the idea of using EPCs as therapeutic agents has grown in popularity.Successful exploitation of EPCs is a complex, multi-step process that includes mobilization, homing to specific sites, adhesion, further differentiation, and functional integration[22].

The first experimental studies for using EPCs as biomarkers of vascular dysfunction in diabetes were done in animal models. A possible role for EPCs in diabetic vascular disease was first investigated in mice. Infusion of human CD34-positive leukocytes, as an EPC-enriched population, was able to accelerate blood flow restoration in diabetic nude mice with experimental hind limb ischemia, but did not have this effect in non-diabetic animals[23]. The reason for the different response of diabetic and non-diabetic mice to the administration of EPCs was not clear, but it could be due to the fact that blood flow restoration in non-diabetic animals was largely provided by physiological ischemia-induced neovascularization, which is hampered in diabetic animals. It is therefore possible that exogenous cells have beneficial effects only in diabetic animals who have either a reduced level or compromised EPC function. Indeed, reduced angiogenic potential of EPCs has been demonstrated in diabetic animals[24].

In type 1 and type 2 diabetic patients, the reduction in circulating EPCs and the functional impairment of cultured EPCs has been reported. Tepper *et al*, showed that peripheral blood mononuclear cells (PBMC)-derived EPCs isolated from type 2 diabetic patients displayed a proliferation rate in culture decreased to control subjects, a weaker adherence to activated human umbilical vein endothelial cells (HUVEC), and a reduced incorporation into vascular structures *in vitro*[20]. Loomans *et al*, reported almost identical results in type 1 diabetic patients[21].

The rate of EPC proliferation from plated PBMCs in diabetic patients was inversely correlated with the levels of glycated hemoglobin, suggesting a possible relation between glucose control and EPC function. Poor adhesion of EPCs to HUVECs demonstrated altered cell-to-cell interactions, which could indicate that EPCs are recruited less avidly *in vivo* at sites of ischemia, as well that re-endothelization by means of bone-marrow derived cells is less likely to take place in the presence of EPC dysfunction. Moreover, Lambiase et al., have shown that poor coronary collateral development (typical for diabetes), may be related to low levels of circulating EPCs[25].

THE MECHANISMS GOVERNING EPCs' ROLE IN DIABETES

Mechanisms underlying the reduction of EPCs in diabetes are largely unknown. Weak bone marrow mobilization, impaired peripheral differentiation, and short survival in peripheral\blood are all candidates. Several mobilizing factors, such as granulocyte colony-stimulating factor (G-CSF), stromal cell derived factor-1 (SDF-1), vascular endothelial growth factor (VEGF), and erythropoietin (Epo) *via* AKT protein kinase pathway activation and endothelial nitric oxide synthase (eNOS), were demonstrated to mediate EPCs' mobilization, proliferation, and migration.

It was revealed that myocardial infarct size in the rat is increased in hyperglycemic conditions, and is associated with a reduced expression of the hypoxia-inducible factors 1 (HIF-1) gene[26]. Chemokine (C-X-C motif) ligand 12 (CXCL12), also known as SDF-1, and its receptor C-X-C chemokine receptor type 4 (CXCR4) both play a critical role in regulating hematopoietic cell trafficking[27]. In non-obese diabetic (NOD) mice, the onset of diabetes is significantly delayed by reducing the level of CXCL12, either by antibody-mediated neutralization or G-CSF-induced suppression of CXCL12 transcription[28,29]. Despite these initial observations, however, how chemokine CXCL12 affects the development of type 1 diabetes has not been fully investigated. Bruhl *et al*, revealed a dose-dependent relation between levels of p21Cip1, that controls cell cycle progression and apoptosis in mature endothelial cells, and levels of circulating EPCs in double and single p12Cip1 knockout mice[30]. In rats with streptozotocin-induced diabetes, circulating EPC levels were reduced, compared to controls and associated with uncoupled eNOS in bone marrow[31].

In particular, it was found that the expression of angiogenic factors VEGF and HIF-1 is reduced in the hearts of diabetic patients during acute coronary syndromes in comparison with control subjects[32]. Moreover, impaired cell-to-cell interactions of EPCs cultured from diabetic subjects could reflect alterations in the so-called "stem cell niche" that hampers cytokine-induced mobilization of stem cells[33]. There is much data supporting the theory that EPCs might decrease because of increased apoptosis. Also, another study shows that EPCs are better protected against oxidative stress than are mature endothelial cells, and therefore it seems unlikely that the decrease in number and dysfunction of EPCs is mediated by increased oxidative stress[34]. Furthermore, EPCs dysfunction in type 2 diabetes patients was associated with oxidative stress due to excessive generation of reactive oxygen species (ROS). It was shown that prolonged exposure of EPCs to high

glucose concentrations *in vitro* increased superoxide anion production, and reduced NO bioavailability[35]. Generation of superoxide anions appears to take place by several processes including glucose auto-oxidation, and increased protein kinase C (PKC) and nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) activity[36]. Moreover, in diabetic patients, the presence of advanced glycation end-products (AGEs) adducts on basement membrane and compromises repair by EPCs with implications for vaso-degeneration during the micro-vasculopathy[37].

NOVEL INSIGHTS INTO THE POTENTIAL THERAPEUTIC USEFULNESS OF EPCS

EPCs have recently generated considerable attention as potential novel diagnostic/prognostic biomarkers for vascular integrity, and therapeutic clinical approaches using these cells are ongoing[38]. There is evidence that some drugs that positively affect vascular function in diabetic patients, could also improve the function and number of circulating EPCs. Thus, it appears that the vasculo-protective effect of these compounds may partly be due to their action on EPCs.

Ohshima *et al*, demonstrated that antioxidant therapy with superoxide dismutase (SOD) in diabetic mice reduced oxidative stress, and increased EPC count and their potential to differentiate into endothelial cells[39]. In addition, a new inhibitor of CXCR4, AMD3100, was found to accelerate blood flow restoration to ischemic tissue in diabetic mice[40]. Also, the treatment with AMD3100 in non-obese diabetic mice abolished T-cell accumulation in the bone marrow and simultaneously inhibited disease development[41].

Notably, it was shown that the angiotensin-converting enzyme (ACE) inhibitors such as ramipril[42], enalapril[28] and *angiotensin* Ⅱ (AT Ⅱ) inhibitors, like valsartan[43] increased EPC levels in patients, probably interfering with the CD26/dipeptidylpeptidase Ⅳ system. Other studies suggested that either the phosphatidylinositol 3-kinase/Akt/endothelial nitric oxide synthase/NO (*PI3K/Akt/eNOS/NO*) signaling pathway or the interaction between hyperglycemia and hyperlipidemia in diabetic patients who have vascular diseases, are potential therapeutic targets for abolishing the impaired function of EPCs[44]. Neutralization of the $p66^{ShcA}$ gene, which regulates the apoptotic response to oxidative stress, prevented high glucose-induced EPC impairment *in vitro* [45]. The existence of molecules acting on EPCs can be used to positively condition cultured EPCs before therapeutic transplantation. Thus, because it is known that chemokine SDF-1α is able to mobilize EPCs, and because EPCs are known to have receptors for SDF-1α, it was demonstrated that SDF-1α - primed EPCs exhibit increased adhesion to HUVEC, resulting in more efficient incorporation of EPCs into sites of neovascularization[46]. Also, it has been shown that platelets promote the homing and differentiation of EPCs at sites of vascular injury[47]. Furthermore, it was hypothesized that circulating microparticles (MPs) are able to program stem/ progenitor cells to repair tissue injury. In particular, it was speculated that MPs of endothelial origin may operate to induce differentiation of *bone marrow*-derived progenitor cells into endothelial cells and subsequently promote postnatal vasculogenesis. Moreover, the treatment with AMD3100 in diabetic patients improved wound healing by correcting EPC mobilization and homing[49]. AMD3100 is now approved for use as a mobilization agent of EPCs in the United States; new data have provided enticing evidence regarding its therapeutic effect in human myocardial infarction[50].(Table 1)

Table 1 Summarizes the potential therapeutic targets to increase EPC number or function

Signaling pathways	Specific drugs
Angiotensin-Convering-Enzyme (ACE)	Ramipril; Enalapril
Angiotensin Ⅱ	Valsartan
PI3-K/Akt/eNOS/NO	Statins
Reactive oxygen species (ROS)	SOD
CXCR4	AMD3100

Another way to improve vascular dysfunction could beby means of a therapy using EPC transplantation. In a very recent study it was demonstrated that administration of circulating human EPCs intravenously had beneficial effects on ischemic brain injury in a mouse model of transient middle cerebral artery occlusion[51]. Transplantation of human cord blood-derived EPCs was reported to contribute to neovascularization in various ischemic diseases, and EPC transplantation on diabetic wounds has a beneficial effect, mainly achieved by their direct paracrine action on keratinocytes, fibroblasts, and endothelial cells, rather than through their physical engraftment into host tissues (vasculogenesis). In addition, an EPC-conditioned medium was shown to be therapeutically equivalent to EPCs, at least for the treatment of diabetic dermal wounds[52].

CONCLUSION

Therapeutic interventions do not necessarily restore a proper endothelial function and, when they do, may improve only some of these variables. Bone marrow-derived circulating EPCs might be a better alternative. For over 10 years, EPCs have been studied as a novel biomarker to assess the severity of diabetes, and as a potential new strategy in regenerative medicine.

Although the role of EPCs in these processes is well established, the challenge for the next decade is to identify and evaluate methods that increase EPC homing and incorporation, thereby enabling targeted delivery of EPCs to a site of interest. This goal might be achieved through the continued characterization of EPCs in animals and humans, coupled with investigations of the long-term potential of EPCs *in vivo*. Once accomplished, the therapeutic potential of this treatment modality could transform the treatment of both cardiovascular disease and diabetes.

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