

Integrative Diabetes and Cardiovascular Diseases

Review Article

Role of Vascular Endothelium in Hypertension, Atherosclerosis and Peripheral Arterial Disease

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Abstract

The vascular endothelium is a dynamic structure which lines the entire circulatory and lymphatic systems and interacts with local and systemic stimuli. It plays an important role in such processes as vasoconstriction/vasorelaxation, inflammation, cell proliferation, and hemostasis. Dysfunction of endothelial cells contributes to the development of different diseases including hypertension, atherosclerosis, and peripheral arterial disease, which are commonly seen in patients with chronic diabetes. Various risk factors including low density lipoprotein oxidation, inflammation, thrombosis as well as imbalance between NO and endothelin production are considered to induce the VE dysfunction and associated cardiovascular diseases. Although several interventions such as thrombolytic agents, anti-inflammatory agents, antioxidants, NO donors, endothelin inhibitors and stem cell therapy are used for the treatment of hypertension, atherosclerosis and peripheral artery disease, none of these have been found to exert satisfactory beneficial effects. Thus a great deal of research work needs to be carried out to define the exact molecular targets and develop newer therapies for the treatment of hypertension, atherosclerosis and peripheral vascular disease.

Keywords: Vascular endothelium; Endothelin-1; Nitric oxide; Inflammation; Sheer stress; Hypertension; Atherosclerosis; Peripheral arterial disease; Pharmacological therapies

Abbreviations: VE: Vascular Endothelium; ECs: Endothelial Cells; HTN: Hypertension; PAD: Peripheral Arterial Disease; PGI₂: Prostaglandin I₂(prostacyclin); cGMP: Cyclic Guanosine Monophosphate; ET-1: Endothelin-1; NO: Nitric Oxide; eNOS: Endothelial NO Synthase; VCAM-1: Vascular Cell Adhesion Molecule-1; EPCs: Endothelial Progenitor Cells; ROS: Reactive Oxygen Species; LDL: Low Density Lipoproteins; HDL: High Density Lipoproteins; CRP: C Reactive Protein; IL: Interleukin; TNF: Tumor Necrosis Factor; LPA: Lysophosphatidyl Acid; HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme A; NAD: Nicotinamide Adenine Dinucleotide; NADPH: Nicotinamide Adenine Dinucleotide Phosphate

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Introduction

Although peripheral arterial disease (PAD) is commonly associated with hypertension and atherosclerosis, the vascular endothelium (VE) has been shown to play a critical role in its pathogenesis. The VE has divergent and unique features as it contributes to the wide variety of functions including fluid filtration, vasomotor tone, mediating the balance between pro- and anti-coagulant activities, neutrophil recruitment as well as nutrient and hormone trafficking [1-4]. More importantly, the VE allows communication of the blood and lymphatic systems with all tissues and organs in the body. Accordingly, when any dysfunction of the VE occurs, either locally or systemically, it may lead to multiple diseases [5,6]. It is pointed out that the VE is an endocrine organ that is able to carry out its function by the participation of membrane-bound receptors that interact with various organ systems [5]. These VE receptors allow cell-to-cell and cell-to-matrix communication and respond to signals from surrounding tissues and blood [5,6]. Various stimuli to the VE include soluble mediators such as proteins, lipid-transporting particles, metabolites and hormones, as well as changes in oxygen concentration, pH, hemodynamic forces and temperature [3]. These stimuli generate responses including modulation of vasomotor tone, vascular permeability, hemostatic balance, inflammatory signals and cellular proliferation [5]. Responses to these stimuli are variable in healthy VE but are attenuated in disease states such as atherosclerosis or diabetes [5].

Endothelial cells (ECs) may occur in activated and dormant states; the dormant ECs represent the resting state and display thrombo-resistant, anti-adhesive and vasodilator phenotype whereas the activated ECs have pro-coagulant, pro-adhesive and vasoconstrictor properties. EC activation results from phenotypic changes that occur when ECs are exposed to physiological and biological stressors. Both phenotypes are in dynamic continuum through the vasculature in the body and the activation at one site may not mean the activation of ECs at another site [7]. The activation of ECs is not an all or nothing response and it is not a sign of a disease state as the normal ECs are very responsive to local stimulation

by the extracellular environment. Stimuli may be either intrinsic such as tumor necrosis factor-alpha, thrombin and lipopolysaccharide or external such as; bacteremia, trauma and shear stress [8]. Thus an extensive research work has been carried out to understand the VE function under both physiological and pathophysiological conditions [1-8]. In the present review, it is intended to discuss the involvement of VE in thrombosis and thrombolysis, interaction with platelets and leukocytes, regulation of vascular tone and cell proliferation, as well as molecular signaling. Furthermore, the role of endothelial dysfunction in the pathogenesis of atherosclerosis, hypertension and PAD is described. In addition, current and potential pharmacological therapies of various cardiovascular diseases targeting endothelial function are discussed.

Physiological Functions of VE

Not only the VE layer serve as a barrier for the protection of smooth muscle cells in the vasculature, it has also been shown to carry out several functions for the maintenance of blood flow. Some of these physiological functions including vasodilatory/ vasoconstricting actions and angiogenesis in which the VE is involved are shown in Figure 1.

Blood flow maintainance

ECs play a crucial role in the regulation of blood flow, specifically through their ability to mediate anti- and prothrombotic properties. Antithrombotic properties of the VE prevent the development of thrombus and the impairment of the antithrombotic properties is usually the consequence of prolonged action of physiological stressors such as inflammation, shear stress, radiation and low flow conditions. The ability of VE to maintain antithrombotic states is due to a multitude of factors starting from the basic make-up of the luminal aspect of VE cells. The luminal side of ECs contains a membrane glycocalyx with negative charged properties preventing similarly charged circulating cells from adhering to its surface [6]. ECs are involved in the synthesis and secretion of antithrombotic compounds that modify and regulate connective tissue components. These compounds exert various effects that range from

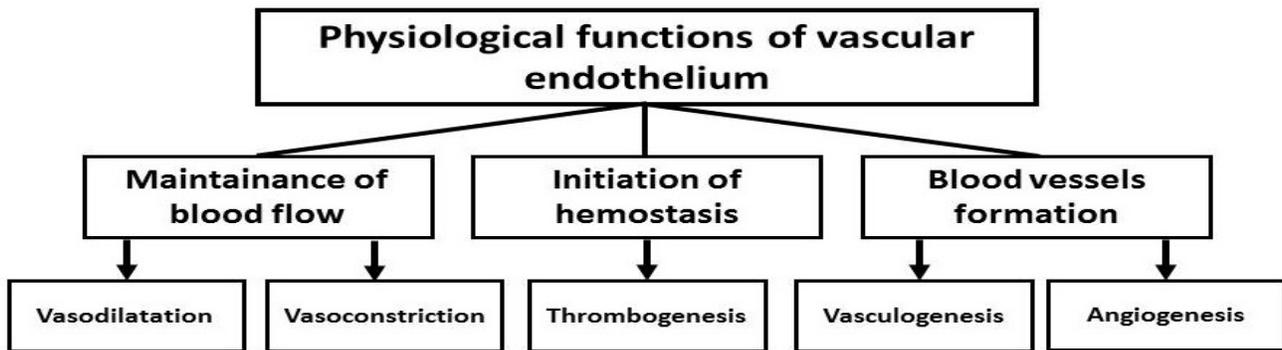


Figure 1: Schematic diagram describing the physiological functions of the vascular endothelium.

vasodilation to vasoconstriction as well as pro- and anti-coagulant functions. All antithrombotic compounds contribute to impairing the development of non-physiological thrombi.

Prostacyclin, also called prostaglandin I₂ (PGI₂), is a potent vasodilator released by ECs in response to biochemical and mechanical signals. Properties of PGI₂ include inhibition of platelet aggregation through the cyclic adenosine monophosphate (cAMP) pathways [9] which directly oppose the effects of thromboxane through interaction at the cell receptor level [10]. Another potent vasodilator released by EC is nitric oxide (NO) which induces relaxation of smooth muscle by increasing the intracellular concentration of cyclic guanosine monophosphate (cGMP) [11]. Release of NO from ECs is a response to such stimuli as thrombin, bradykinin, thromboxane-A₂, histamine, shear stress and platelet aggregation [12]. The presence of NO inhibits the platelet aggregation and the effects of NO are enhanced when accompanied with PGI₂ [13]. The expression of tissue factor on the endothelial surface through endotoxin and cytokine pathways are attenuated by NO, which is important in systemic inflammatory states to maintain blood flow to the peripheral tissues. Antithrombotic mediators of VE are important to maintain homeostasis of the circulatory system. VE cells synthesize and release compounds that activate plasminogen to the serine protease plasmin and are thus involved in fibrin degradation [14]. ECs also have the ability to synthesize heparin-like molecules, which aid in vascular thrombotic resistance

by neutralizing hemostatic proteins [15]. Tissue factor pathway inhibitor-1 is found on the surface of ECs and it is a direct antagonist of the tissue factor as well as factors VIIa and Xa, and has the ability to impair the propagation of the hemostatic cascade. The VE is indirectly involved in the anticoagulant pathways of protein C/protein S. The ability of the VE to prevent thrombosis is paramount in maintaining blood flow throughout the circulatory system, especially in the microvasculature where ECs are the most prominent and the microcirculatory disease is most important [16].

Thrombogenesis and hemostasis

The opposing function to the anticoagulant function of the VE is its prothrombotic function known as thrombogenesis. This is simply described as a complex series of events that form a matrix of platelets, erythrocytes and insoluble fibrin [17]; this deposition of materials creates a mechanical barrier to blood flow. Formation of thrombus varies from the arterial and venous systems by cell composition; arterial thrombi consist of a more tightly packed combination of platelets and venous thrombi are loosely packed with erythrocytes, leukocytes and fibrin. Thrombogenesis and thrombolysis are constantly occurring in the circulatory systems and their regulation is almost entirely dependent on the VE. Factors that can shift this balance to pro-coagulant are important for understanding the disease process in coronary and peripheral vascular diseases. These factors are; alteration in blood flow

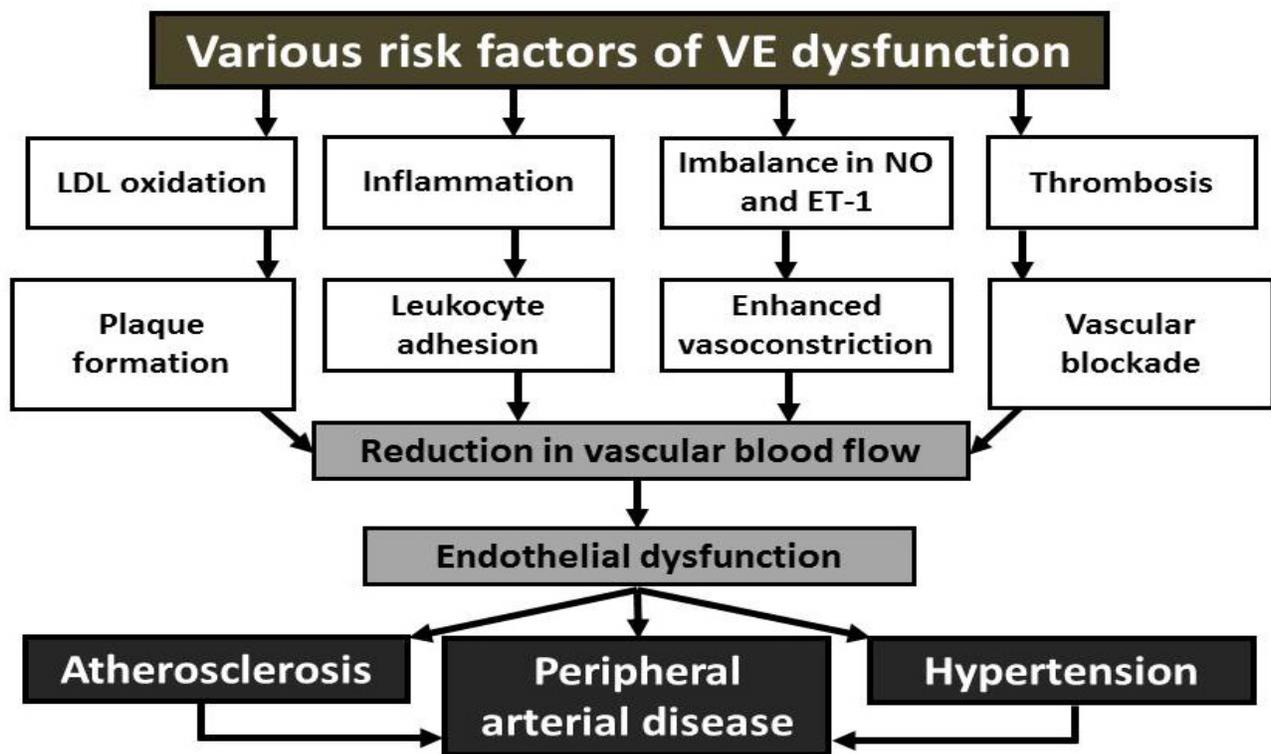


Figure 2: The diagram showing the main factors leading to endothelial dysfunction and development of hypertension, atherosclerosis and peripheral arterial disease.

and shear stress seen in patients who have HTN and deformed vessels secondary to atherosclerotic plaques, calcium deposition and aneurismal disease. The structure of the VE surface contributes to pro-coagulant state when it is broken down by disease processes such as atherosclerosis and diabetes, as well as hyperglycemic states which bring about the glycosylation of proteins in the VE potentiating pro-thrombotic mechanisms [17]. The circulating plasma composition of coagulants contributes to thrombus formation, specifically in genetic states where protein C/protein S pathways are impaired, and in systemic inflammatory states where cytokines impair anticoagulant properties of the VE.

Vasoconstriction and vasodilation

It should be highlighted that the proper function of healthy VE is maintained through the balance between vasodilatory and vasoconstricting agents released from the ECs. The main vasoconstrictor produced by ECs is endothelin-1 (ET-1); it was originally identified in

1988 as the first EC-derived constricting factor [18]. The circulating levels of this short peptide were detected in humans, and it was reported that the circulating level of ET-1 is increased in many cardiovascular diseases suggesting its role in their development [19]. Two receptors for ET-1 (ET_A and ET_B) were identified in 1990 [20,21] giving potential for the use of ET-1 receptor antagonists, such as bosentan, in the treatment of patients with pulmonary arterial hypertension [19]. Two additional isoforms of endothelins (ET-2 and ET-3) were also reported; however, ET-1 is the prominent isoform synthesized by ECs in the vasculature [22]. ET-1 is released continuously from ECs and contributes to the maintenance of vascular tone. It has been observed that ET-1 induces a long-lasting contraction of coronary arteries which is stronger than that of other vasoconstricting peptides such as angiotensin II. Additionally, it has been shown that ET-1 produces constriction of almost all arteries and veins [19].

Nitric oxide (NO), first described in 1980 by Furchgot and Zawadzki [11] as an endothelium-derived relaxing

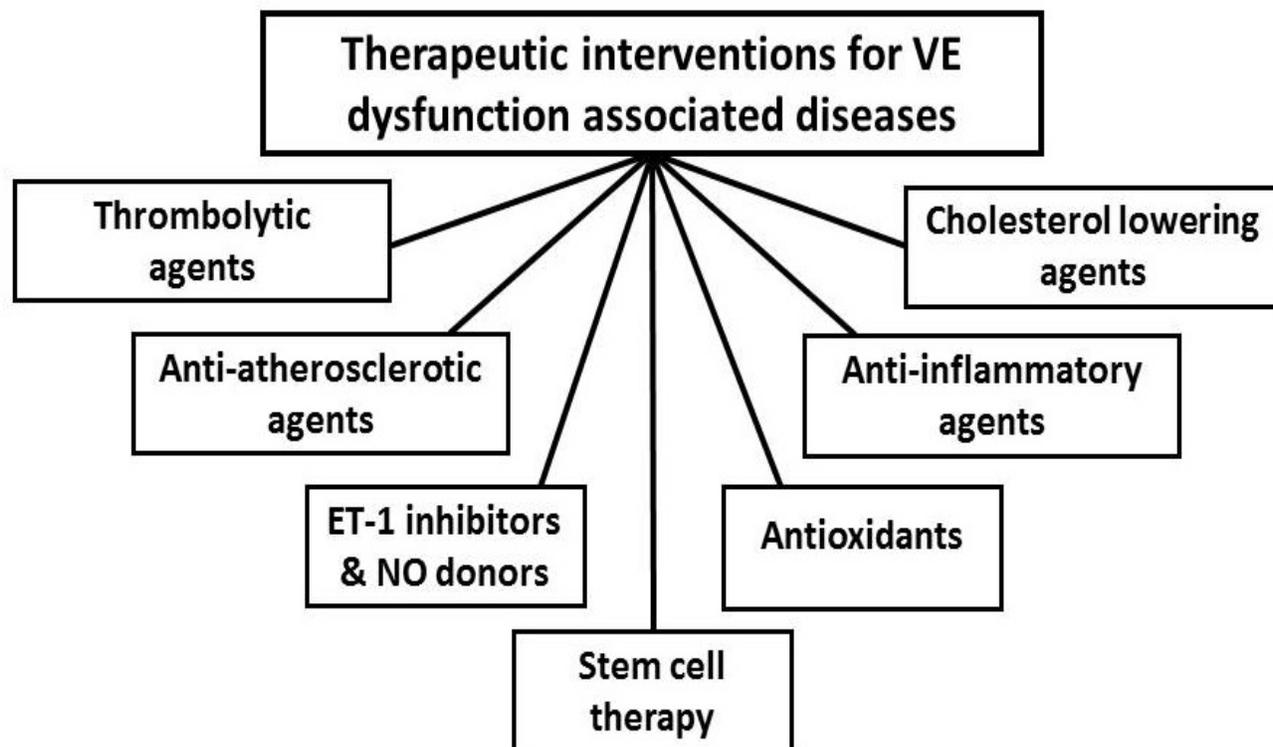


Figure 3: Current and potential therapies in the management of patients with endothelial dysfunction and related cardiovascular diseases.

factor and later identified by Ignarro et al. in 1987 [23], is considered to be one of the important vasodilators participating in the regulation of vascular tone, vascular resistance and blood pressure [24]. Endothelial NO is synthesized from the amino-acid, L-arginine, by constitutively active endothelial nitric oxide synthase (eNOS). Main physiological action of EC-derived NO is the induction of vasorelaxation through an increase in the activity of guanylate cyclase in vascular smooth muscle cells, formation of cyclic guanosine monophosphate (cGMP) and activation of protein kinase G-dependent mechanism resulting in the reduction of intracellular Ca^{2+} concentration [25]. Inhibition of eNOS leads to vasoconstriction which is reversible with administration of NO donors. Additionally, it has been shown that the vasoconstriction caused by NOS blockade can be attenuated by antagonizing ET-1 receptors [26] indicating direct antagonism of NO and ET-1 in maintaining the vascular tone. Taken together, NO and ET-1 are natural vasodilator and vasoconstrictor for the regulation of vascular function, and it is clear that an imbalance between these two mediators of the vascular

tone is a characteristic of endothelial dysfunction and is important for the progression of vascular disease [27].

Platelet activation and inflammation

The VE plays an important role in the activation of inflammatory cells and platelets. Adherence and permeability are two of the major processes that ECs are responsible for when the normal VE is exposed to stimuli such as trauma or infection [28]. Platelet adherence, deposition and activation occur when these come in contact with the disrupted VE. The expression of vitronectin, a receptor on the luminal surface of the VE, is responsible for the adhesion to glycoprotein IIb/IIIa via a fibrinogen bridge found on the platelets [29]. This allows them to aggregate and interact with the VE receptors and proteins. Aggregation occurs through multiple steps including; platelet attachment to collagen and exposure to adhesive proteins, intracellular signaling, and continued expression of platelet receptors. Proteins important in this process are thrombin, collagen, and thromboxane-A2 [29]. Rapidly

enlarging platelet mass represents the primary step in hemostasis and is a normal physiological response to injury of the VE [30]. In VE dysfunction, platelets develop pathological thrombosis which is initiated by platelet adherence and can lead to circulatory compromise in the micro and macro vasculature [31]. Leukocyte adhesion is a major factor in the mobilization of inflammatory cells from the circulation to damaged or infected tissue and it relies on the VE to allow the cells to be picked up from the plasma and subsequent translocation. In disease states such as atherosclerosis, there has been a pathophysiological link made between the EC surface receptor, endothelial-leukocyte adhesion molecule, and an inducible endothelial cell-specific antigen responsible for binding monocytes [32]. The ECs that overlies atherosclerotic lesions express vascular cell adhesion molecule (VCAM)-1 [32] which has been shown to be related to venous thrombosis and protein C deficiency [33]. This is a demonstration that dysfunction of the VE is an initial step in the progression of disease such as atherosclerosis.

Blood vessel formation

The VE plays a vital role in the blood vessel formation and in fact two different processes, namely vasculogenesis and angiogenesis, are intimately involved in the formation of new vessels. While vasculogenesis is a process where mesodermal precursors are differentiated into ECs, angiogenesis is the formation of new vessels from preexisting vessels via proliferation and migration of mature ECs. Different growth factors such as fibroblast growth factor 2 (FGF2) and VE growth factor (VEGF) not only initiate cellular differentiation but also induce endothelial migration and proliferation [34]. In angiogenesis, extracellular proteolysis is essential for disassembly and reassembly of ECs to their environmental matrix and allows their migration to elongate the arterial tree. The angiogenesis is regulated by the balance between pro- and antiangiogenic factors [35]. Recently, it has been suggested that progenitor cells from different origins within the body, termed endothelial progenitor cells (EPCs), may be released into the circulation and contribute to re-endothelialisation. EPCs are also believed to have a potential to induce the new vessel formation in adults (vasculogenesis) without proliferation

and migration of mature endothelial cells (angiogenesis). An extensive research has been carried out to identify how EPCs may be mobilised and contribute to vascular repair, either via neovasculogenesis in ischemic regions or the re-endothelialisation of vessels with endothelial dysfunction [36]. Understanding of these mechanisms would greatly contribute to development of therapeutic strategies based on EPCs administration in the treatment of diseases caused or related to impaired circulation and endothelial dysfunction.

Pathophysiological Functions of VE

The EC dysfunction is a spectrum of alterations in its normal physiological processes; the main processes that become altered are vasoregulatory functions, specifically vasodilatation, pro-inflammatory state leading to hyperadhesiveness for platelets and leukocytes and pro-thrombotic state which impair the blood flow, particularly in the microvasculature [37]. The presence of endothelial dysfunction has been found in coronary artery disease, chronic heart failure, peripheral vascular disease, diabetes as well as chronic kidney failure [30]. In VE dysfunction, an attenuated production and increased destruction of NO has been shown to occur. Oxygen free radicals that are present in post ischemic states usually remove NO from the VE and also damage EC membranes. The damaged membranes then become "leaky" and allow more inflammatory mediators and toxins to pass into the circulation [38]. Some of the risk factors leading to endothelial dysfunction and vascular disease are shown in Figure 2.

Pathogenesis of hypertension

Hypertension (HTN) is a major cause of EC dysfunction as seen when vessels are exposed to increasing wall shear stress and the VE then becomes damaged [39,40]. There is still uncertainty in determining whether the HTN causes the VE damage or the VE dysfunction induces HTN. Originally, it was hypothesized that HTN caused ECs to be in a perpetually activated state leading to deregulation in the variability that is usually seen in various vascular beds [41]. This was not supported when Panza et al. [42] evaluated the effects of treating

HTN with antihypertensive agents, acetylcholine and nitroprusside, and found no improvements in the VE function. This notion was supported by the development of HTN in vascular diseases such as vasculitis or scleroderma renal crisis, where there occurs a damage of the VE with ensuing HTN [43]. In these diseases the elevation of blood pressure is caused by ischemia-induced activation of the renin-angiotensin system rather than the occurrence of hypervolemic state [43]. It has also been shown that when essential HTN was treated with a β -adrenoreceptor blocker, atenolol, and switched to an angiotensin receptor antagonist, irbesartan, it resulted in the correction of persistently altered vascular structure and endothelial function [44]. This supports the idea that when ECs are damaged or become dysfunctional, it can contribute to the development of HTN. Patients with HTN also exhibit less production and response to vasodilator factors; this is often associated with overproduction and enhanced sensitivity to vasoconstrictor peptides and drugs [30]. Taddei et al. [45] suggested that when the VE is chronically exposed to hypertensive states, a decreased availability of NO takes a place as well. A crucial role of NO deficiency in development of hypertension has also been demonstrated in experimental studies using analogues of L-arginine for NOS inhibition leading to hypertension [46]. Additionally, the generation of reactive oxygen species (ROS) occurs, usually secondary to elevated levels of angiotensin-II, leading to an increase in the activity of NADPH oxidase and vascular inflammation [47]. The damaged VE in these states perpetuates the "activated" state of ECs and continues to produce vasoconstrictors, prothrombotic agents and increased membrane permeability. The question as to which came first, HTN or VE dysfunction, is a complex one, but the answer most likely is that both of these states contribute to the each other.

Pathogenesis of atherosclerosis

Patients with atherosclerosis are invariably known to exhibit EC dysfunction. More importantly, risk factors for the generation of atherosclerosis are analogous to the risk factors for the development of endothelial dysfunction. These risk factors include hypercholesterolemia, diabetes, hypertension and cigarette smoking [48]. A particularly important step in

the development of EC dysfunction is brought about by the oxidation of low-density lipoproteins (LDL) [48]. The oxidation of LDLs causes local inflammation in ECs and impairs their ability to regulate their activation, causing a pro-inflammatory and prothrombotic state [49]. This can be attenuated by the use of statins, the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, to correct the presence of hyperlipidemia, which fortuitously increases the bioavailability of NO [50]. An up regulation of VCAM-1, leukocyte adhesion molecule, and increased expression of chemoattractants of leukocytes such as protein-1 appears in dysfunctional ECs [49]. Expression of monocyte adhesion molecules like VCAM-1 allows monocyte uptake and penetration through the vascular wall; these inflammatory cells can cause local destruction through proteolytic enzymes and development of ROS [51]. ROS, such as superoxide radicals, can cause decreased bioavailability of NO in arteries by either degrading it or incorporating it into a cofactor pathway [52]. Inflammatory cell mediators also increase the presence of C-reactive protein (CRP) which has been shown to promote atherosclerosis by directly increasing the translocation of LDLs across the EC and preventing removal of LDLs in the VE walls [53]. CRP has also been shown to contribute to the ROS pool production through the activation of protein kinase C and Src [53]. The translocation of LDLs and interaction with ROS causes enhanced oxidation of LDLs. Oxidized LDLs inhibit the VE production of NO as well as NO effects on the vascular smooth muscles [54]. The oxidized products from the inflammatory processes perpetuate atherogenesis through the release of interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α) from the activated monocytes, ECs and the release of growth factors from smooth muscle cells [55]. Excess of these growth factors and cytokines greatly affect the development of atherosclerosis. ET-1, a potent vasoconstrictor, augments atherosclerosis in multiple ways. ET-1 is a chemoattractant for monocytes and activates macrophages. Activated inflammatory cells release inflammatory mediators such as IL-1, IL-6, IL-8 and TNF- α [56]. Proliferation and chemotaxis of fibroblasts is enhanced by ET-1 as well as migration of smooth muscle cells [57]. The dysfunction of the VE is associated with the instability and eventual rupture of the atheromatous plaque that could be explained due to the lack of antithrombotic function of the damaged

VE [58]. Important player in this process may be lysophosphatidyl acid (LPA) which is released from platelets or produced from phosphatidic acid due to phospholipase A₂ action. LPA is known to cause platelet aggregation and its content in the atherosclerotic plaque is greatly increased in comparison with normal tissue. Thus LPA is suggested to be involved in the development of atherosclerosis and LPA antagonist may prove useful in the treatment of this and related diseases [59]. It should be pointed out that atherosclerosis is considered to develop due to increased inflammation in the VE. The destructive presence of inflammatory cells and mediators contribute to a decrease in the ability of VE to regulate and maintain homeostatic features of the vascular beds throughout the body.

Pathogenesis of PAD

Dysfunction of the VE is important in the development and propagation of PAD in clinical settings and entails impaired production and response to NO. Loss of this response contributes to decrease in oxygen delivery in exercise-induced ischemia when its demand is increased [60]. In the setting of VE dysfunction, the vasoconstrictor effects of catecholamines produced during physical activity are exacerbated resulting in the increase of stenosis, wall shear stress and resistance to blood flow [61]. The response to the decrease in oxygen delivery to the tissue is a compensatory remodeling and collateral formation of the microvasculature [62]. Several studies have shown that for effective remodeling, the NO synthase production as well as NO bioavailability and response must be intact; however this is blunted in VE dysfunction [63,64]. The inability of collateralization and remodeling is a direct example of how important VE dysfunction is in PAD. PAD causes ECs to be in an activated state, perpetuating expression of pro-inflammatory and prothrombotic factors. This activated state of ECs contributes to increased risk of plaque rupture and peripheral ischemia in patients with PAD [65,66]. Plaque rupture is a serious risk factor for critical limb ischemia and limb loss. In the pro-inflammatory state, further propagation of atherosclerosis and impaired healing of the VE occurs. This is responsible for narrowing in the peripheral vessels and decreases NO availability. Additionally, it has been also shown that ET-1 is increased in PAD patients [67] and this seems

to further support the view that there is the progression of PAD. Other vasoconstrictor hormones and peptides can also be seen to induce defects in the VE function and the development of PAD.

Current and Potential Therapies

It is clear that VE dysfunction plays a critical role in development and progression of diseases like HTN, atherosclerosis and PAD. Therefore, targeting the impaired VE function is a way to treat these diseases. There are three main factors that are intimately involved in VE dysfunction and potentially lead to development of associated cardiovascular diseases: (i) imbalance between vasoconstrictors and vasodilators produced by ECs, particularly ET-1 and NO, (ii) enhanced production of pro-inflammatory cytokines, mainly TNF- α and IL-1, and (iii) devastating effects of ROS on the VE. Although HTN, atherosclerosis and PAD are commonly seen in diabetic patients, it is intended to focus the discussion regarding the pharmacological therapies of these cardiovascular diseases separately. Some of the therapies for different cardiovascular diseases associated with endothelial dysfunction are indicated in Figure 3.

Targeting VE in the treatment of hypertension

One of the crucial events leading to VE dysfunction is the imbalance between vasodilator and vasoconstrictor production within ECs, mainly ET-1 and NO. It has been reported that levels of circulating ET-1, an extremely powerful vasoconstrictor involved in vascular remodeling, were increased in most cardiovascular diseases [19]. Identification of ET-1 receptors as a target for pharmacological intervention has led to the discovery of a number of agents that can block the ET receptors. Bosentan is a mixed ET_A/ET_B-receptor antagonist and was the first ET receptor antagonist to be used clinically, particularly in the treatment of pulmonary hypertension [68]. On the other hand, NO donors such as molsidomine or isosorbide mono- or dinitrate have also been successfully used in the treatment of hypertension [69]. Another mechanism by which VE impairment leads to HTN is through the induction of inflammatory processes. However, no

anti-inflammatory drug is used to treat hypertension [70]. Nonetheless, based on pre-clinical studies with different classes of anti-inflammatory agents, immunosuppressant drugs could be used for the treatment of hypertension. It has been demonstrated that mofetil, which blocks T cell and B cell proliferation, reduces the blood pressure in spontaneously hypertensive rats [71], in Dahl salt-sensitive rats [72], as well as in patients with psoriasis and rheumatoid arthritis [73]. Another immunosuppressant, tacrolimus, which blocks T cell activation, is reported to decrease the blood pressure in Dahl salt-sensitive rats [74]. Since the overproduction of ROS damages the VE function and leads to progression of disease, therapeutic potential of common antioxidants such as vitamins A, C, and E, co-enzyme Q, beta carotene, and flavonoids have been studied extensively [75]. A meta-analysis of data on the effects of vitamin C supplementation has indicated reduction in blood pressure [76]; however, large antioxidant clinical trials have failed to demonstrate the beneficial cardiovascular effects [77,78]. Such disappointing results may be explained on the basis that the antioxidant supplementation was too late, too little and too non-specific [79].

Targeting VE in the treatment of atherosclerosis

Since atherosclerotic risk factors such as alterations in lipoproteins, influence the endothelium and lead to the development of atherosclerosis via endothelial dysfunction, the use of statins to correct the presence of hyperlipidemia, which improves the bioavailability of NO [39], is the current strategy for the treatment of atherosclerosis. Interestingly, statins have also been shown to reduce the oxidative stress by blocking the generation of ROS and reducing the NAD⁺/NADH ratio. The antioxidant properties of statins contribute to their protective cardiovascular effects probably independently of their lipid-lowering actions [80]. In addition to statins, high density lipoprotein cholesterol (HDL-C) and paraoxonase 1, an HDL-associated esterase which contribute to the antioxidant and antiatherosclerotic capabilities of HDL-C, seems to be a promising strategy for the treatment of atherosclerosis in the near future. Because HDL-C has been shown to promote endothelial generation

of NO and improve endothelial function and arterial vasoreactivity, these mechanisms can be seen to serve as the basis of anti-atherogenic therapy of HDL [81]. Another mechanism how VE dysfunction leads to development of atherosclerosis is inflammation giving the reason for use of anti-inflammatory drugs for the treatment of atherosclerosis. However, none of the approaches to treat inflammation in order to prevent or reduce atherosclerosis in humans has been successful [82]. It appears that inhibition of a single specific pro-inflammatory cytokine may not reduce atherosclerosis without serious side effects. Thus the aim to prevent or treat atherosclerosis via modulating inflammation remains challenging.

Targeting VE in the treatment of PAD

Different goals for the PAD management are: (i) to decrease the occurrence of cardiovascular events and prevent death, (ii) to reduce limb symptoms, improve exercise capacity, and improve the quality of life and (iii) to prevent or decrease the disability and progression to limb loss [83]. The main strategies in the management of patients with PAD are: lifestyle modifications (reduction of smoking), exercise (walking), diet (low salt, low fat), surgical intervention (revascularization) and pharmacotherapy. Pharmacological interventions include antiplatelet therapies (aspirin), lipid lowering agents (statins) as well as ACE inhibitors (ramipril) and phosphodiesterase inhibitors (cilostazol) [83]. Among all therapeutic strategies currently recommended for the treatment of PAD, antiplatelet therapy with aim to prevent adverse vascular outcomes due to endothelial dysfunction is used. The most common antithrombotic agent in the management of PAD is aspirin [83] and the other antithrombotic agents are heparin-like substances [84]. Additionally, sarpogrelate, a serotonin (5-HT) receptor antagonist, as an anti-platelet, anti-thrombotic and anti-atherosclerotic agent has been found to have beneficial effects in PAD [85]. Another important therapeutic strategy in the management of PAD for improving endothelial function is angiogenesis; the idea of this type of therapy is to use endothelial progenitor cells (EPCs) to induce neovascularization of the diseased area to increase blood flow and thus achieve its better supplementation with oxygen and nutrients. Thus far many clinical trials have investigated

the safety and efficacy of EPCs in the treatment of PAD; the first major study of stem cell therapy in peripheral vascular disease was the Therapeutic Angiogenesis using Cell Transplantation which resulted in improved rest pain, transcutaneous oxygen pressure, and pain-free walking [86]. Improvements in leg pain, walking distance, and ulcer size were maintained during a 2-year follow-up [87]. Additionally, intramuscular stem cell implantation has shown to increase the acetylcholine-mediated endothelium-dependent blood flow suggesting that endothelial dysfunction may be reversible by stem cell therapy in patients with atherosclerotic limb ischemia [88]. Taken together, therapeutic neovascularization improved outcomes in PAD by altering the natural history of this progressive disease via vascular repair and regeneration [89]. Additionally, it has been proposed that stimulation of angiogenesis could be beneficial in the treatment of coronary arterial disease, cardiac failure and tissue injury [90].

Conclusion

The impaired integrity and function of the VE plays a critical role in the development of HTN, atherosclerosis and PAD, which cardiovascular diseases are frequently observed in diabetic patients. We have discussed the reasons for EC dysfunction and its progression to these cardiovascular diseases through EC activation, inflammation and shear stress. The damaged VE is unable to maintain a balance between the activated and dormant states leading to a pro-thrombotic and pro-inflammatory environment in vascular beds throughout the body. Without the VE ability to deal with a stressor effectively, these diseases progress and worsen. Medical therapy has shown to attenuate some of the consequences of the VE dysfunction, including elevated blood pressure, hypercholesterolemia and inflammation. However, without effectively restoring the EC function treatments are usually unsuccessful as the ECs are the key players in maintaining homeostasis of the circulatory system and its communication with body organs.

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