The clinical significance of endothelial dysfunction

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Purpose of review

Endothelial dysfunction is thought to play a pivotal role in the development, progression, and clinical complications of atherosclerosis. Several recent studies have addressed the clinical implications of endothelial dysfunction for cardiovascular events, atherosclerosis, restenosis, and heart failure. Novel findings with respect to endothelial progenitor cells and their alteration by cardiovascular risk factors are characterized and potential therapeutic interventions to improve endothelial and endothelial progenitor cell function are discussed.

Recent findings

Over the past 5 years evidence has accumulated from clinical studies for a close association of the degree of endothelial dysfunction and clinical cardiovascular events in patients with cardiovascular risk factors, coronary disease, acute coronary syndrome, or heart failure. Understanding of the mechanisms leading to endothelial dysfunction has improved, including the notion that dysfunctional endothelial nitric oxide synthase, in part due to deficiency of the endothelial nitric oxide synthase cofactor tetrahydrobiopterin, likely plays an important role. Major progress has been made in understanding the role of endothelial progenitor cells, which likely contribute to both ischemia-induced neovascularization and endothelial regeneration after injury. Endothelial progenitor cell function is altered in patients with cardiovascular risk factors.

Summary

Recent research on endothelial and endothelial progenitor cell dysfunction supports their clinical significance and has led to important insights in the pathophysiology of cardiovascular disease and at the same time provides an important opportunity to develop novel therapeutic approaches. Endothelial function represents a valuable surrogate endpoint to assess the impact of therapeutic interventions.

Keywords

atherosclerosis, endothelial progenitor cells, endothelium, nitric oxide synthase, oxidant stress

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Abbreviations

ACE	angiotensin-converting enzyme
BH4	tetrahydrobiopterin
CHF	congestive heart failure
eNOS	endothelial nitric oxide synthase
HDL	high-density lipoprotein
MI	myocardial infarction

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Introduction

The endothelium plays a crucial role in regulating vascular tone, growth, inflammatory response, coagulation, and thrombocyte adhesion. Common conditions predisposing to atherosclerosis, such as dyslipidemia, hypertension, diabetes, and smoking are associated with endothelial dysfunction, which likely in part explains why these conditions are risk factors and promote the development, progression, and complications of atherosclerosis [1,2].

Endothelial function has largely been assessed as impaired endothelium-dependent vasodilation, in part based on the assumption that endothelium-dependent vasomotion represents a surrogate marker for other important functions of the endothelium. An important rationale for this approach has been the observation that endothelium-derived nitric oxide ($NO^{\bullet}$), synthesized by the endothelial NO synthase (eNOS) from the precursor L-arginine, not only is a major mediator of endothelium-dependent vasodilation but also is critically involved in the regulation of other protective properties of the healthy endothelium [1]. Accumulating clinical studies suggest that impaired endothelium-dependent vasodilation is closely associated with cardiovascular events as described below, thereby further supporting the concept that impaired endothelium-dependent vasodilation reflects important alterations of endothelial function.

In addition, numerous studies have now assessed the mobilization, homing, and function of endothelial progenitor cells (EPCs). Importantly, the major cardiovascular risk factors are associated with impaired function of EPCs *in vitro* that may result in a reduced reendothelialization and neovascularization capacity in these patients. In addition, aging impairs endothelial and EPC function [3••,4•].

Endothelial dysfunction and atherosclerosis

Experimental studies in the late 1990s suggested that endothelial cell NO production prevents endothelial cell adhesion molecule and chemokine expression and prevents thrombocyte activation and aggregation, indicating that endothelial NO may thereby exert important

anti-inflammatory and antithrombotic effects [1,2]. In light of the important role of vascular inflammation for development and clinical complications of atherosclerosis [5], NO may thereby act as an important antiatherogenic molecule. This concept has been supported by observations in eNOS-deficient mice that have a markedly increased endothelial leukocyte adhesion and acceleration of atherosclerotic lesion development [6].

Accumulating clinical studies suggest an important pathophysiological role of endothelial dysfunction, as determined by impaired endothelium-dependent vasodilation, by demonstrating a close association of the degree of coronary or peripheral endothelial dysfunction with cardiovascular events (summarized in [1]). Recently, endothelial dysfunction has been shown to predict future cardiovascular events in patients who have had an acute coronary syndrome [7•]. In addition, impaired flow-dependent, endothelium-mediated vasodilation predicted the occurrence of in-stent restenosis in patients undergoing percutaneous coronary intervention in a recent prospective study [8•]. Moreover, in women with typical angina and evidence of myocardial ischemia but angiographically normal coronary arteries, Bugiardini *et al*. [9•] have shown that after a long-term (10-year) follow-up, endothelial dysfunction was associated with the development of coronary disease and persistence of chest pain.

The clinical relevance of impaired eNOS function is also suggested by studies analyzing eNOS gene polymorphisms. In a recent study Cattaruzza *et al*. [10••] provided evidence that an eNOS polymorphism, the CC type of the ^{786}C/T single-nucleotide polymorphism in the promotor region of eNOS causing impaired shear stress sensitivity of eNOS expression, is more frequent in patients with coronary disease, providing evidence that reduced shear stress–dependent eNOS activation may contribute to coronary disease.

Interestingly, in a recent large-scale study of more than 2000 healthy young adults, the number of cardiovascular risk factors was correlated with increased carotid intima–media thickening in subjects with an impaired endothelium-dependent vasodilation, but not in subjects with an enhanced endothelium-dependent vasomotion [11••], suggesting that the status of systemic endothelial function may modify the association between risk factors and atherosclerosis.

Endothelial dysfunction may not only promote vascular inflammation, but conversely systemic inflammation can induce endothelial dysfunction [12]. This has been convincingly demonstrated in a recent large-scale study of 600 children with acute infection, who had a substantially impaired endothelium-dependent vasodilation during acute infections [13••]. The association of acute infections with endothelial dysfunction may in part explain the recent observation that there is a substantial increase in cardiovascular events after an acute pulmonary or urinary infection [14]. Furthermore, inflammation-induced endothelial dysfunction may provide, at least in part, an explanation for the recent observation that patients with rheumatoid arthritis, a systemic inflammatory disease, have a markedly increased risk for cardiovascular events [15].

Endothelial dysfunction and heart failure

Accumulating experimental data have suggested that impaired eNOS-derived NO availability not only may lead to impaired vasomotion in patients with congestive heart failure (CHF) but also has other important pathophysiological implications, such as increased left ventricular remodeling and dysfunction in heart failure.

Although modulation of cardiac function by NO is complex, recent experimental studies have provided evidence that both endothelial and cardiomyocyte-specific overexpression of eNOS result in improved left ventricular function following myocardial infarction (MI) [16,17]. Moreover, statin treatment improved left ventricular function and survival following MI in wild-type but not in eNOS-deficient mice, further suggesting an important role of eNOS for left ventricular function and survival following MI [18•].

Accumulating clinical studies suggest a close association of endothelial dysfunction with an impaired prognosis in patients with heart failure. In a study of 259 subjects with both ischemic and nonischemic CHF, both decreased flow-dependent, endothelium-mediated vasodilation and decreased exhaled NO production were associated with an increased risk of death or urgent transplantation after adjustment for other known CHF prognostic factors [19•]. In two further studies, impaired peripheral endothelial function was independently associated with adverse long-term outcome in patients with heart failure [20•,21].

Interestingly, in patients with decompensated heart failure a particularly severe impairment of endothelium-dependent vasomotion, likely resulting from increased oxidant stress, has been observed [22] that recovers to some extent after recompensation, raising the possibility that endothelial dysfunction may directly contribute to decompensated heart failure.

Potential role of endothelial progenitor cells

After the initial report by Asahara *et al*. [23] interest has been substantial in understanding the mobilization, homing, and function of EPCs in various conditions. Circulating EPCs may represent an important endogenous repair mechanism to maintain the integrity of the endothelial monolayer and promote ischemia-induced neovascularization. It is now understood that cardiovascular risk factors

and aging impair either the number or in-vitro function of EPCs [4•,24]. The underlying mechanisms and pathophysiological consequences of this EPC dysfunction are the subject of intensive research. Interestingly, the mobilization of EPCs by vascular endothelial growth factor, statins, estrogen, or physical exercise has been shown to be dependent on eNOS [18•,25–27]. Moreover, EPCs from eNOS-deficient mice had a reduced neovascularization capacity in the hind limb ischemia or retina ischemia model [25,28], suggesting that eNOS-dependent signalling is important for EPC function.

The homing of EPCs includes adhesion, chemoattraction, and transmigration of EPCs [24]. Several mediators involved in this process have now been identified. β_2-Integrins have been shown to mediate adhesion of EPCs in postnatal vasculogenesis [29], whereas adhesion of EPCs for reendothelialization of denuded vessels is partially mediated by vitronectin receptors [30]. The chemoattractant stromal cell–derived factor 1 likely plays an important role for homing in ischemic tissue [31] and the protease cathepsin L has been demonstrated to be important for EPC invasion and ischemia-induced neovascularization [32].

A first clinical study suggests that a reduced number of circulating EPCs as assessed by flow cytometry analyses ($CD34^+$, KDR^+ cells) is associated with cardiovascular events, supporting an important role for endogenous vascular repair to modulate the clinical course of vascular disease [33•].

Novel mechanistic insight into endothelial dysfunction

Increased vascular reactive oxygen species production and accumulation of the endogenous NO synthase inhibitor asymmetric dimethylarginine (ADMA) have been suggested as major mechanisms causing endothelial dysfunction [1,34,35]. In particular, it has now been recognized that under conditions in which endothelial levels of tetrahydrobiopterin (BH4), an essential cofactor of eNOS, are deficient, eNOS becomes dysfunctional and produces superoxide rather than NO, which has been termed 'eNOS uncoupling'. Thus, eNOS may have two faces in atherosclerosis and likely in heart failure depending on tissue BH4 metabolism. Uncoupled eNOS has been associated with accelerated atherosclerotic lesion formation, which could be prevented by BH4 treatment [36]. Conversely, increased endothelial BH4 synthesis by targeted transgenic guanosine triphosphate–cyclohydrolase I overexpression has been shown to reduce endothelial dysfunction and atherosclerosis in apolipoprotein E knockout mice [37•].

In patients with heart failure, Dixon *et al.* [38] have provided evidence for dysfunctional eNOS in thrombocytes. Moreover, it has recently been observed that pressure load–induced myocardial remodeling was partially attributable to reduced BH4 and increased eNOS-dependent reactive oxygen species generation [39].

Thus, understanding of the regulation of vascular BH4 levels is an important area of research with the potential for novel therapeutic strategies.

Interventions to improve endothelial function

The vascular endothelium has emerged as an attractive therapeutic target, given accumulating data suggesting an important role of endothelial dysfunction for prognosis in vascular disease. Several approaches that have been shown to improve endothelial function are discussed here.

Life style changes

The recent INTERHEART study has underscored the important role of life style for cardiovascular risk [40]. In this respect it has been shown that weight reduction improves endothelial function in overweight adults [41] and in young obese children [42•]; however, diet combined with exercise training was more effective [42•].

Exercise training has been shown to improve endothelial function in patients with coronary disease or heart failure in the forearm, as well as in the coronary circulation [43]. Recent studies have provided mechanistic insights into the mechanisms whereby exercise training may improve endothelial function. Adams *et al.* [44•] have shown that exercise training reduces vascular oxidant stress and nicotinamide adenine dinucleotide phosphate oxidase activity in patients with coronary disease. These findings correspond well with experimental data, indicating that a sedentary life style is associated with increased oxidant stress, NADPH oxidase, and endothelial dysfunction as compared with a physically active life style [45•].

Statins

Statins rapidly improve endothelium-dependent vasomotion in humans [46]. In a recent study, we observed that simvastatin, but not ezetimibe, therapy improved endothelial function and increased EPCs in patients with CHF, suggesting that prolonged statin treatment can exert beneficial effects on endothelial function independent of lipid lowering [47•].

Inhibition of angiotensin-converting enzyme inhibitor and angiotensin-1 receptor blockade

Angiotensin-converting enzyme (ACE) inhibition and angiotensin-1 receptor blockade have been shown to exert beneficial effects on endothelial function [48], and recently angiotensin-1 receptor blockade has been shown to increase circulating EPCs [49]. First studies suggest that combined statin and ACE inhibitor or angiotensin-1 receptor blocker treatment is more effective in improving endothelial function than single therapy [50,51]; however,

more studies in different patient populations are needed to address the effect of combined treatment.

High-density lipoprotein cholesterol

There is an increasing interest in understanding the effects of high-density lipoprotein (HDL) cholesterol on vascular function. HDL has recently been shown to stimulate eNOS, partly through binding to scavenger receptor, class B type I, which is expressed in endothelial cells [52] and the lysophospholipid receptor sphingosine-1-phosphate $(S1P)_3$ [53]. Acute administration of HDL has been shown to improve endothelial function in hypercholesterolemic men [54]. Furthermore, in mice, administration of human HDL increased myocardial perfusion that was dependent on eNOS, since it was not observed in eNOS-deficient mice [55]. Therefore, interventions that increase HDL have the potential to improve endothelial function and myocardial perfusion.

Acyl-CoA: cholesterol acyltransferase

There are additional potential targets in lipid metabolism that may be important for the regulation of endothelial function. The Acyl-CoA:cholesterol acyltransferase (ACAT) enzymes, discovered about 7 years ago, mediate intracellular esterification of cholesterol. In a first clinical study it has been shown that ACAT inhibition improved resistance-vessel endothelial function in hypercholesterolemic subjects, with small effects on circulating cholesterol [56].

Conclusion

Accumulating data suggest a close association of endothelial dysfunction and possibly EPC levels with cardiovascular events, supporting the concept that alterations in endothelial and EPC function play an important pathophysiological role and promote development and complications of vascular disease. Current strategies to reduce cardiovascular events in patients at risk, including life style changes such as weight loss and increased physical activity as well as pharmacologic interventions with statins, ACE inhibitors, or angiotensin-1 receptor antagonists, are associated with improved endothelial function. Novel strategies to improve endothelial function may therefore have the potential to improve prognosis in vascular disease.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 593–599).

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