Potential Mechanisms of Impaired Endothelial Function in Arterial Hypertension and Hypercholesterolemia

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This review focuses on the role of impaired endothelial function for the development of atherosclerosis in human arterial hypertension and hypercholesterolemia in vivo. Potential mechanisms underlying impaired endothelial function and decreased bioavailability of nitric oxide under these clinical conditions are discussed. It further addresses therapeutic strategies aimed at improving the bioavailability of nitric oxide in these patients. The overall conclusion is that the bioavailability of nitric oxide is probably impaired, not by a single defect, but by various mechanisms affecting nitric oxide synthesis as well as nitric oxide breakdown. In both diseases increased superoxide anion production and oxidative stress represent a major mechanism. Decreased bioavailability of nitric oxide not only impairs endothelium-dependent vasodilation, but also activates other mechanisms that play an important role in the pathogenesis of atherosclerosis. Thus, therapeutic strategies should aim to restore bioavailability of nitric oxide, which has been demonstrated for lipid-lowering therapy in hypercholesterolemia and blood pressure control in hypertension. In addition, antioxidative strategies will represent a major therapeutic tool against atherosclerotic diseases in the future. Statins and blockers of the renin-angiotensin system seem to have such antioxidative effects independent from their effects on lipid profiles or blood pressure control.

Introduction

In 1980 Furchgott and Zawadzki [1] discovered that the effect of acetylcholine and other substances on the contractile state of smooth vascular muscle cells was dependent on the presence and integrity of endothelial cells. Further studies have established the importance of the vascular endothelium for determining vascular tone through the production and release of different vasodilator and vasoconstrictor substances that control the activity of the underlying smooth muscle layer. The most important endothelium-derived vasodilating substance was found to be nitric oxide (NO) [2]. NO is generated from the amino acid L-arginine by endothelial NO synthase (NOS III) [3]. This enzyme is stimulated by blood flow across the endothelial cell surface (shear stress), or by chemical mediators, such as acetylcholine, which stimulate receptors on the endothelial cell membrane. NO is produced and released both tonically and stimulated under the influence of endothelial agonists. NO diffuses to the underlying smooth muscle cells stimulating soluble guanylate cyclase to generate cyclic GMP, which causes smooth muscle relaxation and therefore endothelium-dependent vasodilation (Fig. 1) [4].

The term *endothelial function* is widely used for the ability of the endothelium to cause stimulated "endothelium-dependent vasodilation." The secretion of vasodilating factors, especially NO, represents one of the key functions of the vascular endothelium. Besides its vasodilating effects, NO has been found to be a principal factor involved in the antiatherosclerotic properties of the endothelium (Fig. 2) [5]. NO interferes with key events in the development of atherosclerosis, such as monocyte and leukocyte adhesion to the endothelium [6] and platelet aggregation and adhesion [7]. NO also decreases endothelial permeability [8] and inhibits vascular smooth muscle cell proliferation [9]. In accordance with these findings, inhibition of the NO-producing enzyme NOS caused accelerated atherosclerosis in experimental models [10]. The term *endothelial dysfunction* evolved in the scientific literature in order to conveniently label the above-mentioned alteration of vascular endothelial function. Although this term is somewhat imprecise, it has become widely used.

Endothelial dysfunction is often present before structural irregularities in the arterial wall become apparent [11]. Thus, a relative deficiency in local NO availability seems to represent a final common pathway that accelerates atherogenesis in humans. In patients with arterial hypertension, hypercholesterolemia, diabetes mellitus, smoking, and also heart and renal failure, impaired endothelial function as a consequence of impaired bioavailability of nitric oxide has been identified in coronary and peripheral arteries [12].

Basic problems in assessing endothelial function in human blood vessels in vitro and in vivo and the difficulties

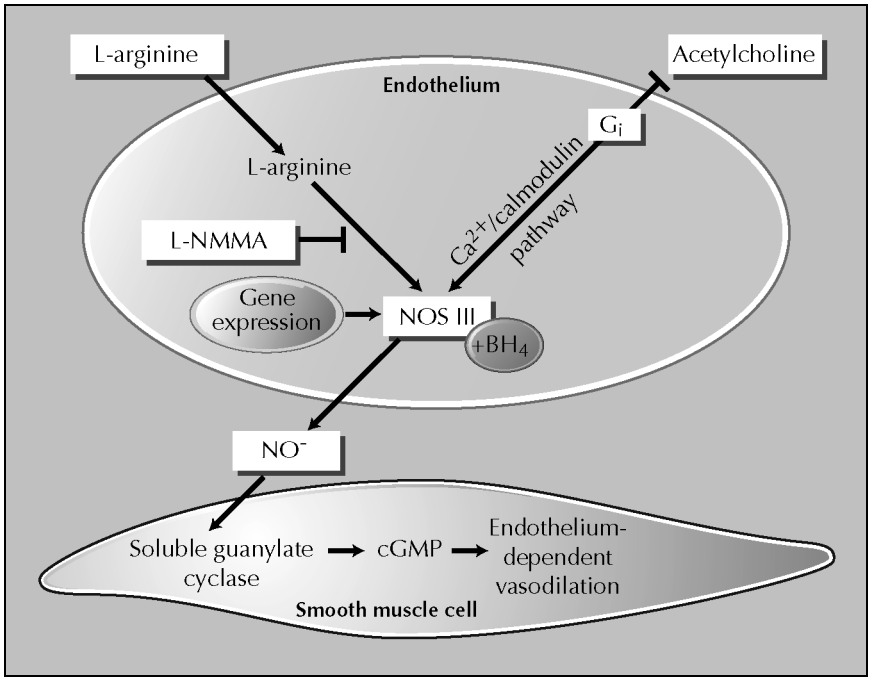

Figure 1. Mechanisms of endothelium-dependent vasodilation: the L-arginine/nitric oxide (NO) pathway. Endothelial NO synthase (NOS III) can be stimulated by acetylcholine through the muscarinic receptor. The signal transduction pathway includes the receptor G_i complex and the Ca^{2+}/calmodulin pathway. NOS III can be blocked by the L-arginine analogue N^G-monomethyl-L-arginine (L-NMMA). NO is produced in the presence of the NOS cofactor tetrahydrobiopterin (BH_4). NO stimulates soluble guanylate cyclase to produce cGMP, which induces endothelium-dependent vasodilation in the smooth muscle cell.

in extrapolating observation obtained in isolated arteries to the intact human circulation have been reviewed previously [13]. However, the assessment of endothelial function by measuring endothelium-dependent responses in different vascular beds permits the clinically orientated scientist to investigate the role of the endothelium in local vasomotor control under physiologic conditions and in the genesis of vascular disease in his patients [14].

This review focuses on impaired endothelial function in vivo in human arterial hypertension and hypercholesterolemia. Potential mechanisms underlying impaired endothelial function and the prognostic significance of endothelial dysfunction are discussed, as are evolving therapeutic strategies under these conditions.

Assessment of the Nitric Oxide–cGMP Pathway

In humans the vasodilator response of coronary or peripheral arteries to pharmacologic agents can be measured using angiography for the coronary circulation or forearm plethysmography for the peripheral forearm circulation. Because endothelium-dependent vasodilation can be easily assessed in the human forearm, with only minimal risk for the patient, data have been obtained in the human forearm vascular bed [14]. Of note, a close relation of endothelium-dependent vasodilation between coronary and peripheral arteries has been demonstrated [15], despite the fact that forearm vessels rarely develop clinical atherosclerosis. In arterial hypertension and hypercholesterolemia, several investigators have independently demonstrated that hypertensive patients have abnormal stimulated endothelium-dependent vasodilation of the peripheral and coronary vasculature [16–20].

The prognostic significance of coronary endothelial dysfunction for the development of coronary atherosclerosis has been demonstrated in several studies [21,22•]. In addition, it has been shown that forearm endothelial dysfunction is a marker of future cardiovascular events in patients with essential hypertension [23••].

To assess the contribution of the NO–cGMP pathway to the abnormal endothelium-dependent vasodilation, the vascular effects of the NOS inhibitor N^G-monomethyl-L-arginine (L-NMMA) have been studied under baseline conditions and during stimulated endothelium-dependent vasodilation. In normal subjects, infusion of L-NMMA induces vasoconstriction [4], indicating that continuous production and release of NO participates in the regulation of vascular tone under physiologic conditions. Furthermore, L-NMMA blunts the response to acetylcholine but not to exogenous administration of nitrovasodilators in normal subjects [4], indicating that the vasodilator effect of acetylcholine is at least in part mediated by the stimulated release of NO.

In hypertensive or hypercholesterolemic patients, the infusion of L-NMMA does not significantly modify the response to acetylcholine [4,24] when compared with healthy controls. The reduced vascular effects of L-NMMA in these patients indicate an impaired production and release of NO in arteries of hypertensive or hypercholesterolemic patients.

These findings, however, do not allow an assessment of whether impaired endothelial function in these patients is a consequence of diminished synthesis of endothelium-derived NO, or of normal synthesis but enhanced breakdown of NO before it reaches the vascular smooth muscle cell. Figure 3 schematically depicts some possible mechanisms that might account for the decreased biologic availability of NO.

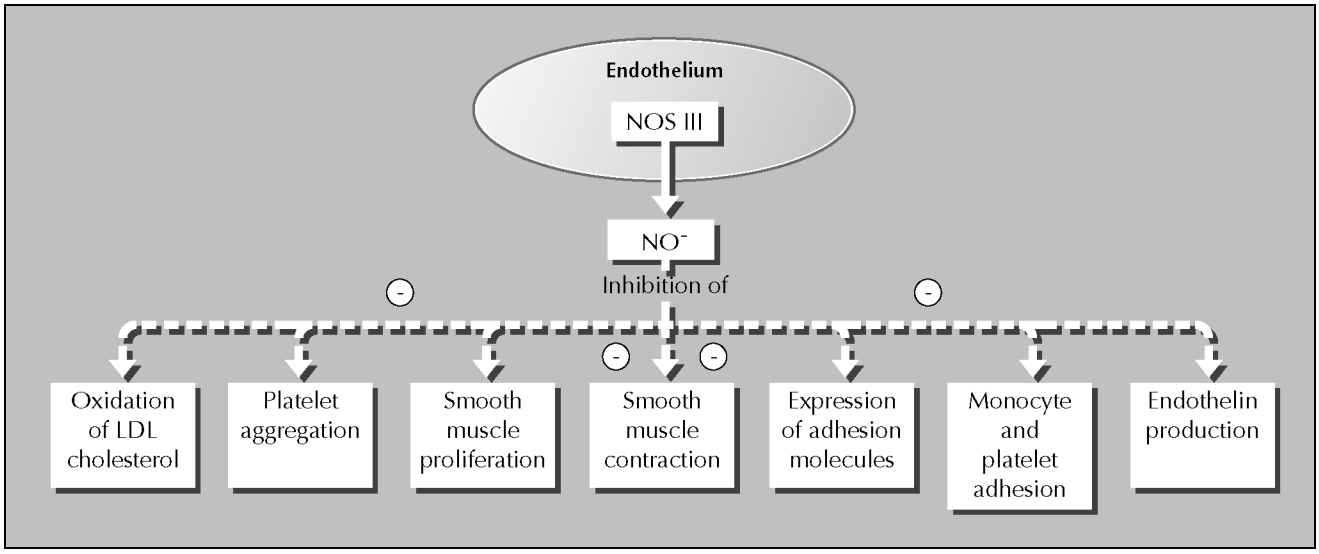

Figure 2. Antiatherosclerotic properties of nitric oxide (NO) produced by the vascular endothelium. LDL—low-density lipoprotein; NOS—nitric oxide synthase.

Diminished Synthesis of Nitric Oxide?

Asymetric dimethylarginine and endothelial dysfunction

Diminished bioavailability of NO impairs endothelium-dependent vasodilation and activates mechanisms that may play an important role in the pathogenesis of atherosclerosis. Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of NOS that can modulate NO production [25]. Plasma levels of ADMA have been shown to be elevated in hypercholesterolemia [26] and hypertriglyceridemia [27], and clinical studies have demonstrated an association between increased plasma ADMA concentrations and hypertension [28•]. In addition, a direct relationship between plasma ADMA levels and carotid intima media thickness in healthy humans has been reported [29], and an association between plasma ADMA levels and endothelium-dependent brachial artery vasodilation in young hypercholesterolemic individuals has been shown [26]. In hypercholesterolemia, the infusion of L-arginine improves endothelium-dependent vasodilation [30], suggesting that decreased availability of the substrate for NOS might be responsible for the impaired vascular responses in these patients. The biologic mechanisms behind the increased ADMA concentrations are not yet fully understood, but reduced degradation by oxidative mechanisms may be involved in its accumulation [31]. Together, ADMA seems to play an important role in the regulation of NO bioavailability in hypertension and hypercholesterolemia, and it has been suggested to be a novel marker of atherosclerosis.

Reduced nitric oxide synthase activity?

Elevated or modified low-density lipoprotein (LDL) cholesterol is a major cause of injury to the endothelium. There is evidence of reduced transcription and enhanced breakdown of NOS transcripts with increasing concentrations of oxidized LDL cholesterol [32]. Long-term stimulation with oxidized LDL may also lead to a decrease in the amount of the NOS III protein through induction of cytokines [33], which are elevated due to the inflammatory process of the arterial wall in atherosclerosis. Endothelial inflammation plays a major role in the development of atherosclerosis [5]. It increases the formation of hydrogen peroxide and free radicals such as superoxide anion and hydroxyl radicals in plasma [34]. These substances that are cytotoxic to endothelial cells may reduce the formation of NO either through a direct injurious effect on endothelial cells with the consequence of less synthesis of NO, or through an enhanced breakdown of NO.

Enhanced Breakdown of Nitric Oxide?

The in vivo half-life of NO is determined mainly by its reaction with oxyhemoglobin and superoxide anion (O_2^-) [35,36]. The reaction of superoxide and NO occurs at a diffusion-limited rate with the production of the powerful oxidant peroxynitrite ($ONOO^-$). Inactivation of NO by O_2^- limits the bioavailability of NO and leads to nitrate tolerance, vasoconstriction, hypertension, and accelerated atherogenesis.

An increased production of superoxide radicals has been directly assessed by chemiluminescence in the aortic wall of cholesterol-fed rabbits [37,38]. In addition, chronic treatment with superoxide dismutase, a scavenger of free radicals, has been proven effective in restoring endothelium-dependent vasodilation in animals with hypercholesterolemia and hypertension [39,40].

In human essential hypertension, intrabrachial infusion of vitamin C, a scavenger of oxygen free radicals, increased the response to acetylcholine in coronary and peripheral

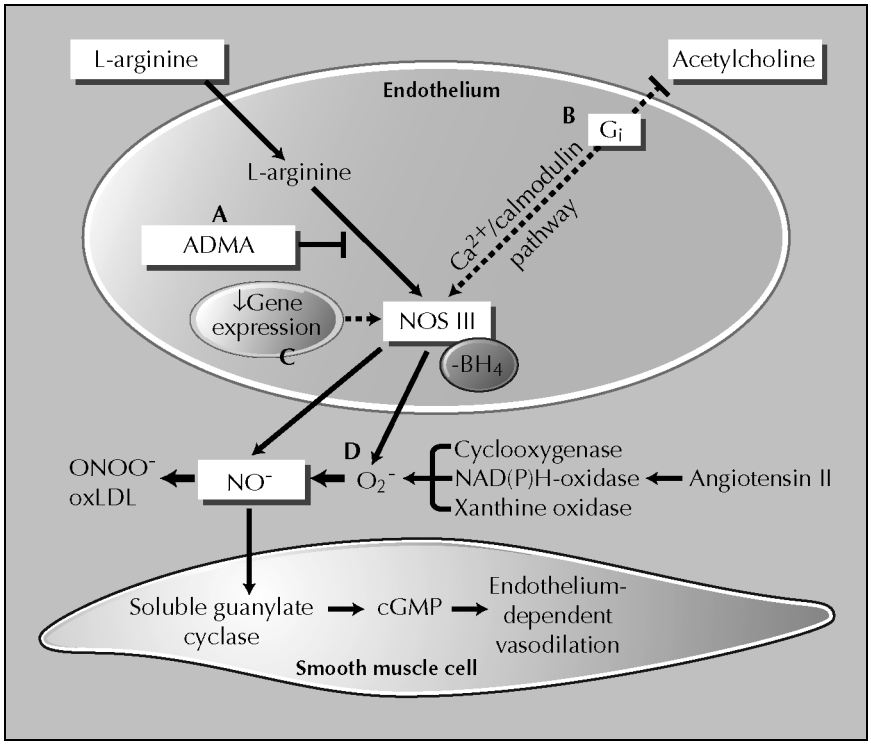

Figure 3. Potential mechanisms that may disturb endothelial nitric oxide (NO) bioavailability: diminished NO synthesis due to substrate deficiency or/and competitive inhibition of endothelial NO synthase (NOS III) by endogenous asymmetric dimethylarginine (ADMA) (labeled *A*); disturbances in signal transduction related to the receptor/G-protein complex or the Ca^{2+}/calmodulin pathway (labeled *B*); reduced activity of NOS III (labeled *C*); and enhanced breakdown of NO due to enhanced superoxide production (labeled *D*). Superoxide may be generated by NOS III itself (especially if the cofactor tetrahydrobiopterin [BH_4] is depleted), xanthine oxidases, the cyclooxygenase pathway, or NAD(P)H-driven oxidases that can be stimulated by angiotensin II via AT_1 receptors. Superoxide anion scavenges NO to produce peroxynitrite ($ONOO^-$). Superoxide can also lead to the formation of oxidized low-density lipoprotein (oxLDL).

arteries [41] and restored the inhibiting effect of L-NMMA, whereas the responses to sodium nitroprusside remained unchanged in hypertensive patients [42]. In parallel to findings in essential hypertensives, it has been demonstrated that coadministration of vitamin C with acetylcholine improves endothelium-dependent vasodilation in hypercholesterolemic patients [43].

Thus, inactivation of NO through increased superoxide anion production seems to represent a major mechanism for impaired endothelial function in arterial hypertension and hypercholesterolemia. With increasing age or underlying cardiovascular risk factors, the production of proatherosclerotic oxidative stress increases and the availability of the antiatherosclerotic substance NO decreases simultaneously in the vessel wall. The balance of these two substances seems to determine the severity of the resulting vascular damage (Fig. 4). Superoxide anions may be generated by different enzymatic and nonenzymatic sources, like the xanthine oxidase system, the cyclooxygenase system, the NOS itself, or angiotensin II mediated through NADPH-dependent oxidases.

Cyclooxygenase system

Blockade of cyclooxygenase with indomethacin resulted in augmentation of the vasodilator response to acetylcholine in hypertensive patients but not in normotensive controls. In addition, the positive effect of vitamin C on endothelium-dependent vasodilation is no longer observed in the presence of indomethacin [42]. Thus, it is conceivable that the cyclooxygenase system may represent a source of superoxide anion production.

Increased nitric oxide synthase III superoxide production and cofactor BH_4 depletion?

Based on in vitro experiments, it has been proposed that enhanced superoxide production may be caused by NO synthase itself. This is supported by observations showing that both de-endothelialization [37] and infusion of the selective NOS antagonist nitro-L-arginine [44] could not only prevent NO formation, but could also inhibit increased formation of oxygen radicals. In other words, NOS III is both an NO- as well as a superoxide-producing enzyme. In the presence of a deficiency of tetrahydrobiopterin (BH_4), an essential cofactor of NOS [45], uncoupling of the L-arginine–NO pathway is observed, which results in increased formation of oxygen radicals by NOS in vitro [46]. In hypercholesterolemic patients, it has been demonstrated that the administration of BH_4 restored the impaired endothelium-dependent vasodilation in theses patients [47]. These observations suggest that NOS III may have a dual role in the pathogenesis of atherosclerosis: under physiologic conditions, it generates low concentrations of NO and probably peroxynitrite, which favor an antiatherosclerotic environment. However, in hypercholesterolemia, it may contribute to the formation of oxidative stress by a reduction in BH_4-dependent NO formation and an unopposed superoxide formation by the enzyme. The potential role of BH_4 depletion with the consequence of increased superoxide production by endothelial NOS III has not yet been examined in patients with arterial hypertension. However, this potential mechanism might also be important for the increased superoxide production that has been demonstrated in hypertensive patients.

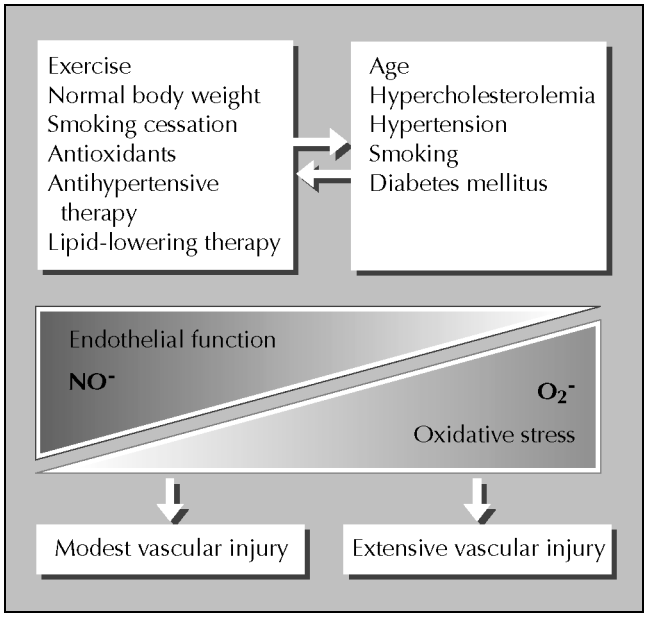

Figure 4. Changes of the balance between oxidative stress and endothelial function/nitric oxide (NO) bioavailability in the vascular wall as induced by age or typical cardiovascular risk factors or interventions known to reduce the incidence of cardiovascular events. The balance between NO and O_2^- determines the degree of vascular injury.

Angiotensin II-induced superoxide generation

Elevated angiotensin II activity, which is strongly correlated with hypertension, is a major trigger of endothelial dysfunction in hypertensive patients. Angiotensin II via AT_1 receptor stimulation activates NAD(P)H-dependent oxidases, a very important vascular source of superoxide anion, to generate reactive oxygen species in the endothelium, smooth muscle cells, and the adventitia of blood vessels, leading to endothelial dysfunction, cell growth, and inflammation [48]. An upregulation of AT_1 receptor gene expression by LDL cholesterol associated with increased NAD(P)H-dependent vascular superoxide production and impaired endothelium-dependent vasodilation has become evident during the past few years [49–51]. The cholesterol-induced upregulation of AT_1 receptor density on vascular smooth muscle cells suggests a novel mechanism by which hypercholesterolemia could be involved in the onset and progression of cardiovascular diseases. Through attenuating O_2^- production, angiotensin II aggravates arterial hypertension by reducing NO availability, which in turn leads to endothelial dysfunction, accelerated progression of atherosclerosis, ischemic heart disease, renal failure, and cerebrovascular disease.

Therapeutic Implications

Impaired bioavailability of NO in hypercholesterolemia and arterial hypertension not only exerts negative effects on vascular tone, but also inhibits and activates those mechanisms that play an important role in atherogenesis, such as platelet aggregation [7], vascular smooth muscle cell proliferation and migration [9], monocyte adhesion, and expression of adhesion molecules [5,6]. Thus, lipid-lowering or antihypertensive drug therapy should aim to improve endothelial function by restoring NO availability and by reducing oxidative stress.

Lipid-lowering therapy

Recent large clinical trials have demonstrated that significant reductions in LDL cholesterol with statins decrease morbidity and mortality rates associated with coronary artery disease as well as with stroke [52,53]. Because serum cholesterol levels are strongly associated with the development of atherosclerotic disease, it as been initially assumed that cholesterol reduction by statins is the predominant mechanism underlying their beneficial effects in cardiovascular disease. However, the benefit of statins occurs early in the course of statin therapy and subgroup analysis of large clinical trials indicates that statin-treated individuals have less cardiovascular disease than placebo-controlled individuals with comparable serum cholesterol levels [54]. Subsequently, it has been suggested that these drugs may have antiatherogenic effects in addition to their capacity to lower lipids and lipoproteins [55]. In fact, intriguing experimental data have shown that statins exhibit pleiotropic effects beyond their lipid-lowering actions, including enhancement of endothelial NO production [56], inhibition of smooth muscle proliferation, and anti-inflammatory and antioxidative actions [57].

A number of recent studies have shown, that cholesterol-lowering therapy improves endothelium-dependent vasodilation and the bioavailability of NO in coronary and peripheral arteries, and that this effect may explain the reduced incidence of adverse coronary events known to result from cholesterol-lowering therapy [58,59,60•]. Although LDL cholesterol and superoxide production are clearly associated with endothelial dysfunction and reduced availability of NO, therapy with statins may improve endothelial function through other mechanisms independent of their lipid-lowering effects. In fact, there is increasing experimental evidence that statins have additional beneficial antiatherosclerotic effects by acting directly on endothelial cells. In this context, statins increase NO availability by stimulating and upregulating endothelial NOS in vitro independent of their cholesterol-lowering actions [56]. Another potential mechanism by which statins may favorably affect the endothelium and attenuate endothelial dysfunction is through their antioxidant effects. Statins enhance endothelium-dependent vasodilation by inhibiting the production of reactive oxygen species, such as O_2^- and hydroxy radicals, from aortas of cholesterol-fed rabbits, although plasma total cholesterol levels were not different from control animals [61]. Although lipid lowering by itself can lower vascular oxidative stress, some of these antioxidant effects of statins appear to be cholesterol independent. For example, statins attenuate angiotensin II-induced free radical production in vascular smooth muscle cells by inhibiting NADH oxidase activity and downregulating angiotensin type 1 receptor expression [62,63•]. In vitro, direct measurement of diffusible NO, together with current

measurements of O_2^-, proved that in the presence of statins the NOS system operates with high efficiency toward increasing NO activity by activation of NO release and by concurrent inactivation of O_2^- [64•].

Recently, we demonstrated that short-term lipid-lowering therapy with statins is able to improve endothelial function and NO availability almost completely after 3 days in hypercholesterolemic patients, probably by decreasing oxidative stress [65]. This improvement seems to be more rapid than the accompanying decline in LDL cholesterol and not related to these changes in lipid profiles. Our findings therefore suggest a lipid-independent effect of statins in humans.

Antihypertensive therapy

Animal studies have convincingly shown that normalization of blood pressure can improve endothelial function. However, the results in human hypertension are more inconsistent. In patients with essential hypertension, tonic basal release of NO can be improved by reductions in blood pressure if they are induced by an angiotensin-converting enzyme (ACE) inhibitor or by a calcium antagonist [66]. However, stimulated endothelium-dependent vasodilation is unaffected when blood pressure per se is reduced by a composition of various antihypertensive drugs [67]. These findings suggest that blood pressure reductions can restore tonic but not stimulated NO release. Thus, it has been suggested that the potential for antihypertensive drugs to improve stimulated endothelial function is dependent on their ability to counteract those mechanisms that impair endothelial function (*eg*, oxidative stress) and not on their blood pressure–lowering effect per se. However, any effects of antihypertensive therapy on endothelial function will still be confused with the blood pressure–lowering effect of these compounds, as long as they are not compared with a control group treated with a blood pressure–lowering agent theoretically not affecting the endothelium.

Studies testing the effects of long-term ACE inhibition on vascular remodeling and endothelial dysfunction in hypertensive patients have shown that after treatment with ACE inhibitors, but not with the β-blocker atenolol, the vasodilator response to acetylcholine could be restored [68]. Similar findings could be obtained for treatment with the AT_1 receptor antagonist losartan [69], irbesartan [70], or candesartan [71] compared with atenolol in small resistance arteries. As mentioned above, in animal experiments angiotensin II increases the production of superoxide via membrane-bound NAD(P)H driven oxidases. This effect is associated with impaired relaxation to acetylcholine. Also, under experimental conditions, it has been demonstrated that losartan can normalize vascular superoxide anion production and relaxation to acetylcholine [72]. Interestingly, even in patients with hypercholesterolemia, angiotensin II receptor antagonism can improve endothelial dysfunction by diminishing oxidative stress [73•]. Thus, AT_1 receptor antagonists could exert a beneficial effect on endothelial function by diminishing superoxide production and reducing NO breakdown in patients with arterial hypertension, as well as in patients with hypercholesterolemia. Furthermore, it can be speculated that in the presence of an AT_1 receptor antagonist, angiotensin II may bind to unblocked angiotensin II type 2 receptors, which may even stimulate NO synthesis in endothelial cells and in coronary microvessels [74]. Most recently, antihypertensive therapy with valsartan was found to improve endothelial function, an effect that was not seen in controls on diuretic therapy with comparable blood pressure reduction [75].

Most investigations on β-adrenergic receptor antagonists showed that atenolol or propanolol do not beneficially affect endothelial function compared with calcium antagonists or angiotensin II blocking agents [69]. However, the vasodilating β-blockers nebivolol or carvedilol may exert a vasodilating effect on forearm circulation through activation of the L-arginine/NO pathway in essential hypertension [76], probably by inhibition of the generation of reactive oxygen species [77].

Calcium antagonists have been shown to be effective in reversing endothelial dysfunction of angiographically normal and stenotic epicardial coronary vessels in essential hypertension [78]. In agreement with these results, the calcium antagonists lacidipine and nifedipine also show a beneficial effect on endothelial function in the forearm microcirculation [79,80]. Therefore, these data indicate that calcium antagonists are effective in reversing endothelial dysfunction in essential arterial hypertension in different vascular beds probably by an additional antioxidant activity. Experimental data indicate that calcium antagonists exert an antioxidant effect and therefore could protect endothelial cells against free radical injury and diminish oxidative breakdown of NO [81]. In the presence of nifedipine, vitamin C no longer increases either the vasodilation to acetylcholine or the inhibiting effect of L-NMMA in hypertensive patients [80]. Thus, nifedipine seems to improve endothelial dysfunction in essential hypertension by restoring NO availability with a mechanism probably related to antioxidant activity.

Conclusions

Decreased bioavailability of NO has been identified as a major cause of impaired endothelial function. Bioavailability of NO is probably impaired not by a single defect, but by various mechanisms affecting NO synthesis as well as NO breakdown. Increased superoxide anion production and oxidative stress represent major mechanisms for impaired endothelial function. Most of the available clinical and experimental data support the concept that an alteration in the redox balance in endothelial cells leads to disturbances of vascular integrity and the subsequent development of atherosclerosis.

Impaired bioavailability of NO in hypercholesterolemia and arterial hypertension not only exerts negative effects on vascular tone but also inhibits and activates those mechanisms that play an important role in the pathogenesis of atherosclerosis, such as platelet aggregation, vascular smooth

muscle cell proliferation and migration, monocyte adhesion, and expression of adhesion molecules. Thus, lipid-lowering or antihypertensive drug therapy should aim to improve endothelial function by restoring NO availability to exert beneficial effects on the development of atherosclerosis. Increasing data exist on additional antioxidative effects of statins, ACE inhibitors, AT_1 receptor antagonists, and calcium antagonists. These additional effects may provide further antiatherosclerotic properties of these drugs besides their main lipid- or blood pressure–lowering action.

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